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METABOLISM AND CEREBRAL ENERGY STATE: EFFECT OF ACUTE HYPERAMMONEMIA IN BEAGLE DOG

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Abstract—The acute effect of hyperammonemia (NH_4^+ blood level 0.2 mM) was evaluated in the isolated dog brain *in situ*. The interference of the transmethylating system of *S*-adenosyl-L-methionine was also studied by means of infusion with *S*-adenosyl-L-methionine or adenosine (blood level 0.4 mM).

The changes induced by hyperammonemia on the cerebral *glutamate-ammonia system* (pyruvate, α-oxoglutarate, oxaloacetate, L-alanine, L-glutamate, L-aspartate, L-glutamine, NH_4^+) were evaluated. Cerebral detoxication of ammonia is connected with the formation of glutamine and, to a lesser extent, of alanine, and is balanced by a decrease in aspartate; glutamate, oxaloacetate, pyruvate and α-oxoglutarate are unmodified or slightly modified.

Cerebral *intermediate metabolism of glucides* was largely activated by acute hyperammonemia, a marked increase in Gibbs free energy being observed. A fraction of this energy not exceeding 10 per cent can be ascribed to the synthesis of glutamine. Hyperammonemia induced a variation of the *resting transmembrane potential* (as indirectly obtained by applying the Nernst equation), which becomes less negative.

The glutamate-ammonia system is the most directly involved in the detoxication of NH_4^+ during hyperammonemia syndromes. In fact, the basic event of this process consists in the conversion of a carboxyl acid into an amino acid, with ammonia uptake. The balance of the cerebral ammonia-detoxicating power must be evaluated by taking into account the changes occurring in the various components, as a function of the amount of ammonia reaching the cerebral tissue.

Some participants in the glutamate–ammonia system are intrinsically connected with the intermediate metabolism of glucides. The relationships between glycolysis or Krebs' cycle intermediates and the glutamate–ammonia system require that the investigations be extended to the study of hyperammonemia effects also at the level of cerebral energy metabolism.

The function of cerebral cells requires a specific and uneven distribution of ions across the membranes. When present extracellularly NH_4^+ (like K^+) decreases the resting transmembrane potential, therefore bringing the potential closer to the threshold for firing and causing a general increase in nerve-cell excitability [1–3]. Consequently, it is also important to evaluate the effect induced by hyperammonemia on the resting transmembrane potential.

The glutamate–ammonia system interferes with other biological systems of the brain, e.g. the *S*-adenosyl-L-methionine transmethylating system. Indeed, *S*-adenosyl-L-methionine: is found under an activated form; it releases adenosine as a metabolic product; during its metabolic transformation it yields L-cysteine (which, by condensating with α-oxoglutarate, yields mercaptopyruvate and glutamate); it is involved in the transmethylation processes affecting also catecholamines.

In the present study *in vivo*, acute hyperammonemia was induced by artificially raising the blood NH_4^+ concentration by infusion of ammonium acetate to the regional cerebral circulation. In order to eliminate response variations due to the different blood levels of the various components, the cerebral blood flow was kept constant during each trial. The technique of *in situ* isolated brain perfusion was used since it allows the proper composition of perfusing blood, blood flow rate, blood oxygenation to be selected at will [4–14].

By the technique of the dog brain isolated *in situ*, in this work we have investigated the effect at cerebral level of the acute hyperammonemia syndrome: (1) on the ammonia-detoxicating power; (2) on fuels, end-products and intermediates of the glycolytic pathway and citric acid cycle; (3) on the resting transmembrane potential. Moreover, we have studied the interference at cerebral level of the perfusion of *S*-adenosyl-L-methionine or adenosine on the effect of the hyperammonemia syndrome, evaluated by the three groups of parameters indicated above.

MATERIALS AND METHODS

The experiments were carried out on female beagle dogs (aged 240–360 days and weighing from 12.5 to 15.6 kg) all kept under the same environmental conditions (22 ± 1°, relative humidity = 60 + 5 per cent) and fed only a standard diet (Altromin Rieper) with water *ad lib*. The animals were pre-anaesthetized with urethane (0.4 g/kg i.p.), and the anaesthesia was induced and maintained only during the surgical procedure by chloralose (20 → 40 mg/kg i.v.). The animals were immobilized by intravenous injection of gallamine triethiodide (2–3 mg/kg), and artificially ventilated. Because anaesthetics affect cerebral energy charge, EEG pattern was used to determine the removal of anaesthetic activity before the start of the experiment on cerebral metabolism.

The operative procedure consisted mainly [4, 6, 11, 12] in the isolation of the external jugular vein and the common carotid arteries, with ligature of all their branches (except the internal carotid arteries) and of the vertebral vessels. Both of the isolated jugular veins were ligated, cannulated, and connected to the venous reservoir of the pump–oxygenator system (through the gravitational flow). Both of the isolated carotid arteries were also cannulated and connected to the pump–oxygenator system. The electroencephalogram as well as both systemic pressure and cerebral perfusion pressure were recorded on a 12-channel polygraph (Physioscript EE12—Schwarzer). A 2.5 cm diameter hole was made in the frontoparietal area; a plastic funnel was fitted into the hole and the skin was tightly sutured around the funnel; subsequently, the plastic funnel was sealed with a rubber stopper and thermally insulated.

The brain perfusion apparatus employed consisted of a venous reservoir, an oxygenator with a gasmeter, a roller-type pump with a flowmeter, two blood filters (polyester staple 1622 Montefibre), a perfusion pressure regulator with a manometer, a blood exchanger with a telethermometer, and an apparatus to eliminate blood foam [4, 6, 12]. Prior to the extracorporeal perfusion, the pump–oxygenator system was filled with 500 ml of heparinized compatible blood, filtered through polyester staple and adjusted to pH 7.35 using 1 M sodium bicarbonate. A flow of a $O_2 + CO_2$ mixture (95:5), maintained at the rate of 5 l min^{-1}, was passed into the oxygenator during the extracorporeal brain perfusion. The blood flow rate was kept between 41 and 43 ml min^{-1}, the pressure being equal to the initial systemic pressure of the animal.

Induction of acute hyperammonemia. At the end of the preparative procedure and after an equilibration period, the hyperammonemia syndrome was induced in the dogs by infusion of ammonium acetate into the extracorporeal circuit at the initial concentration of 2×10^{-4} M. The level of NH_4^+ into the perfusion arterial blood was kept constant by the continuous infusion of 6.5 μmoles min^{-1}, during the 20 min of the syndrome.

To study the interference of *S*-adenosyl-L-methionine transmethylating system on acute hyperammonemia, 20 min before inducing the hyperammonemia an infusion of *S*-adenosyl-L-methionine or adenosine was started. *S*-adenosyl-L-methionine or adenosine were present in the perfusion circuit at the initial concentration of 4×10^{-4} M; during the 40 min of the experiment, they were continuously infused (1 μmole min^{-1}).

Our investigation was focused on three series of biological events taking place in brain tissue:

(1) the NH_4^+-detoxicating power of the glutamate–ammonia system;

(2) the brain energy balance related to the intermediate metabolism of glucides;

(3) the resting transmembrane potential, indirectly obtained by applying the Nernst equation.

In order to define the effect induced by the hyperammonemia syndrome, control values were taken as base values, changes (Δ') in the parameters examined being calculated by difference. For statistical analysis, the Student *t*-test was used in all instances ($P < 0.05$).

Analytical methods. For the evaluation of the arteriovenous differences of metabolites across the brain, glucose, glutamate, glutamine, alanine, O_2 and NH_4^+ uptake, and lactic acid formation were evaluated from simultaneously drawn arterial and venous blood samples. By continuous infusion of glucose (25 μmoles min^{-1}) to the venous reservoir, the glucose concentration in the blood was kept at a constant level. For the evaluation of metabolites in brain, at the set time the motor area of the cortex was frozen *in situ* by pouring liquid nitrogen into the plastic funnel fitted into the cranial vault. The cortical portion of the frozen brain was cut and removed using a rotating cold hollow tube during continuous irrigation with liquid nitrogen. The frozen cerebral tissue was then immersed into liquid nitrogen for 10–15 min and quickly (3–4 sec) powdered by a precooled automatic apparatus (Microdismembrator Braun) using frozen 1·23 M perchloric acid. The subsequent steps of the analytical procedure were carried out in a cooled box at 0–5° until a perchlorate-free extract was obtained [15]. Metabolites were determined by enzymatic techniques: alanine [16]; NH_4^+ [17]; aspartate [18]; citrate [19]; glycogen [20]; glucose [21]; glutamate [22]; glutamine [23]; lactate [24]; malate [25]; α-oxoglutarate [26]; O_2 [27]; pyruvate [28]; ATP [29]; ADP [30]; AMP [30], phosphocreatine [31]. The oxaloacetate concentration was calculated from the formula:

$$[\text{oxaloacetate}] = \frac{[\text{pyruvate}] \times [\text{malate}]}{[\text{lactate}]} \times (K_{MDH} \cdot K_{LDH})$$

where K_{LDH} and K_{MDH} are the equilibrium constants of *lactate dehydrogenase* and *malate dehydrogenase*, respectively [32].

Evaluation of the NH_4^+-detoxicating power of the glutamate–ammonia system. This evaluation was obtained from the balance of variations in the arteriovenous levels and/or in the cerebral concentrations of NH_4^+, carboxyl acid (α-oxoglutarate, pyruvate, oxaloacetate) and amino acids (L-glutamate, L-alanine, L-aspartate, L-glutamine).

The calculated changes (Δ') induced by ammonium acetate were: (i) Δ'CU = change in *Cerebral Uptake* (nmoles g^{-1} min^{-1})

where:

Δ'CU = CBF × ΔAV; CBF = Cerebral Blood Flow (ml min^{-1} g^{-1}); ΔAV = ArteroVenous differences (nmoles ml^{-1}) of metabolites across the brain;

(ii) Δ'CTC = change in *Cerebral Tissular Concentration* (nmoles g^{-1} min^{-1}) $\Delta'CTC = (CTC_{amm} - CTC_{cont}) \cdot t^{-1}$ where: CTC_{amm} = cerebral tissular concentrations (nmoles g^{-1}) of metabolites after ammonium acetate perfusion; CTC_{cont} = cerebral tissular concentrations (nmoles g^{-1}) of metabolites in control conditions; t = time (min); (iii) Δ'CMR = change in *Cerebral Metabolic Rate* (nmoles g^{-1} min^{-1}) Δ'CMR = Δ'CU − Δ'CTC; (iv) $\Delta'CMZR_{NH_4^+}$ = change in *Cerebral Metabolizing Rate for* NH_4^+ (nmoles g^{-1} min^{-1}) $\Delta'CMZR_{NH_4^+} = \Delta'CTC_{glutamate} + \Delta'CTC_{aspartate} + \Delta'CTC_{alanine} + 2\Delta'CTC_{glutamine}$; (v) $MPI_{NH_4^+}$ = *Metabolizing Power Index* for NH_4^+ $^{MPI}_{NH_4^+} = (\Delta'CMZR_{NH_4^+})/(\Delta'CMR_{NH_4^+})$.

Computation of cerebral energy change by NH_4^+. This evaluation was obtained from the balance of variations in the arterovenous levels and/or in the cerebral concentrations of fuels (e.g. glucose, glycogen), of intermediates (e.g. pyruvate) and end-products (e.g. lactate, oxaloacetate) related to the cerebral metabolism of glucides.

The calculated changes (Δ') induced by ammonium acetate were:
(i) $\Delta'CU$ = change in *Cerebral Uptake* (nmoles g^{-1} min^{-1}) $\Delta'CU = CBF$ (ml min^{-1} g^{-1}) $\times \Delta AV$ (nmoles ml^{-1}); (ii) $\Delta'CTC$ = change in *Cerebral Tissular Concentration* (nmoles g^{-1} min^{-1}) $\Delta'CTC = [CTC_{amm}(\text{nmoles } g^{-1}) - CTC_{cont}(\text{nmoles } g^{-1})]$. t^{-1}(min); (iii) $\Delta'CMR$ = change in *Cerebral Metabolic Rate* (nmoles g^{-1} min^{-1}) $\Delta'CMR = \Delta'CU - \Delta'CTC$; (iv) $\Delta'CAnMR_{glucose}$ = change in *Cerebral Anaerobic Metabolic Rate for the glucose* units (nmoles g^{-1} min^{-1})

$$\Delta'CAnMR_{glucose} = -0.5\ \Delta'CMR_{lactate}$$

where:

$\Delta'CMR_{lactate}$ = change in *Cerebral Metabolic Rate for lactate*. In fact each mole of glucose (or of glucosyl unit derived from glycogen) anaerobically yields 2 moles of lactate; (v) $\Delta'CAcMR_{glucose}$ = change in *Cerebral Aerobic Metabolic Rate for glucose* (nmoles g^{-1} min^{-1}) $\Delta'CAeMR_{glucose} = \Delta'CMR_{glucose} + \Delta'CMR_{glycogen} - \Delta'CAnMR_{glucose}$; (vi) $\Delta'[\Delta G^\circ]$ = change in the *Cerebral free energy* (mcal g^{-1} min^{-1}).

In fact, for the measurement of the energy change induced by NH_4^+ in the brain system, we used the function ΔG or *Gibbs free energy*. To further simplify actual numerical computations, we used the *standard* free energy change ($\Delta G'^\circ$), assuming the value of -47 kcal mol^{-1} for the reduction of glucose to lactate, and the value of -686 kcal mol^{-1} for the oxidation of glucose to H_2O and CO_2. The computated values of free energy change should be regarded as *relative* and utilized only for the evaluation of the changes (Δ') occurring between the control condition and the NH_4^+-induced condition: $\Delta'(\Delta G'^\circ)$.

Computation of the resting transmembrane potential. For the indirect calculation of the resting transmembrane potential (E_h) we used the Nernst equation:

$$E_h = -\frac{RT}{F} \ln \left(\frac{[K^+]_{int} + \alpha[NH_4^+]_{int}}{[K^+]_{ext} + \alpha[NH_4^+]_{ext}} \right) \text{mV}$$

where: R = gas law constant (1.987 cal mol^{-1} deg^{-1}); T = absolute temperature; F = Faraday constant (23,061 cal·vol^{-1} $equiv^{-1}$); $\alpha = 0.2$.

The subscripts *int* and *ext* refer to intracellular and extracellular concentrations, respectively; α is the permeability of NH_4^+ relative to K^+ (2). The blood $[NH_4^+]$ and $[K^+]$ were used as an approximation of those existing in brain extracellular fluid [33]. In this case, the NH_4^+-induced variations in the resting transmembrane potential as calculated by the Nernst equation [33] agree with actual direct measurements made on cat motor neurons and squid giant axons [2, 3].

RESULTS

Ammonium acetate (0.2 mM in the cerebral perfusing blood) causes a derangement in the components of the cerebral *glutamate–ammonia system* (Table 1), i.e. a significant increase in glutamine and alanine, and a significant decrease of both aspartate and oxaloacetate, while glutamate, pyruvate and α-oxoglutarate remain unchanged. As shown in Table 2, ammonia detoxication is brought about by glutamine and, to a lesser degree, by alanine. Concurrently, the detoxicating role of glutamate and aspartate becomes less important, probably due to the amidation of glutamate to glutamine.

Table 2 shows that adenosine perfusion (0.4 mM) does not remarkably modify the ammonium-detoxi-

Table 1. Action of ammonium acetate (added to the cerebral perfusing blood: 2×10^{-4} M) on some components of the *glutamate–ammonia system* evaluated in cerebral tissue and by arterovenous differences across the brain

Parameters	Unit of measurement	Control conditions (*a*)	Perfusion with ammonium acetate after and during the infusion with: (*b*)	*S*-Adenosyl-L-methionine (4×10^{-4} M) (*c*)	Adenosine (4×10^{-4} M) (*d*)
Cerebral blood flow	ml min^{-1} g^{-1}	0.494 ± 0.011	0.492 ± 0.010	0.477 ± 0.012	0.485 ± 0.015
NH_4^+ uptake	nmoles min^{-1} g^{-1}	2 ± 1	78 ± 2●	94 ± 3●▲	81 ± 3●
Glutamate uptake		<1	<1	<1	<1
		From arterovenous differences across the brain			
Glutamine uptake		1.2 ± 0.3	1.6 ± 0.4	1.1 ± 0.3	<1
Alanine uptake		1.1 ± 0.6	<1	1.5 ± 0.4	<1
NH_4^+		0.23 ± 0.04	0.42 ± 0.01●	0.44 ± 0.02●	0.39 ± 0.03●
Glutamate		10.20 ± 0.41	10.09 ± 0.32	10.12 ± 0.11	10.06 ± 0.06
Glutamine		5.43 ± 0.08	6.29 ± 0.02●	6.51 ± 0.12●	6.39 ± 0.07●
Alanine		0.435 ± 0.011	0.585 ± 0.011●	0.595 ± 0.007●	0.635 ± 0.017●▲
		Concentration in brain			
α-Oxoglutarate	μmoles g^{-1}	0.199 ± 0.013	0.192 ± 0.006	0.202 ± 0.003	0.195 ± 0.002
Aspartate		2.44 ± 0.10	1.93 ± 0.04●	1.80 ± 0.06●	1.80 ± 0.10●
Pyruvate		0.100 ± 0.008	0.104 ± 0.004	0.102 ± 0.003	0.103 ± 0.002
Oxaloacetate	nmoles g^{-1}	3.3 ± 0.2	2.7 ± 0.1●	2.5 ± 0.1●	2.8 ± 0.1●

* The values are means (± standard errors) of six preparations of beagle dog. The symbol (●) indicates the statistical significance as compared with control condition: (*b*), (*c*) and (*d*) versus (*a*). The symbol (▲) in the hyperammonemic animals indicates the statistical significance as compared with dogs not infused by *S*-adenosyl-L-methionine or adenosine: (*c*) and (*d*) versus (*b*). Level of statistical significance: $P < 0.05$.

Table 2. Changes (Δ') induced by ammonium acetate (added to cerebral perfusing blood: 2×10^{-4} M) on the *glutamate–ammonia system* of the brain as compared with control condition

Cerebral infusion with	Metabolite	Δ'CU	Δ'CTC	Δ'CMR	Δ'CMZR$_{NH_4^+}$	Metabolizing Power Index for NH_4^+
		nmoles g^{-1} min^{-1}				
	NH_4^+	+76	+9.5	+66.5		
	Glutamate	<0.5	−5.5		−5.5	
	Glutamine	<0.5	+43.0		+86.0	
	Alanine	<0.5	+7.5		+7.5	
	Aspartate		−25.5		−25.5	
	Pyruvate		<0.5			
	α-Oxoglutarate		<0.5			
	Oxaloacetate		<0.5			
(*a*)	Total				+62.5	0.94
	NH_4^+	+92●	+10.5	+81.5●		
	Glutamate	<0.5	−4		−4	
	Glutamine	<0.5	+54●		+108●	
S-Adenosyl-L-methionine	Alanine	<0.5	+8		+8	
	Aspartate		−32●		−32●	
	Pyruvate		<0.5			
	α-Oxoglutarate		<0.5			
	Oxaloacetate		<0.5			
(*b*)	Total				+80●	0.98
	NH_4^+	+79	+8	+71		
	Glutamate	<0.5	−7		−7	
	Glutamine	<0.5	+48		+96	
Adenosine	Alanine	<0.5	+10		+10	
	Aspartate		−32●		−32●	
	Pyruvate		<0.5			
	α-Oxoglutarate		<0.5			
	Oxaloacetate		<0.5			
(*c*)	Total				+67	0.94

* The calculated parameters are: Δ'CU = change on Cerebral Uptake; Δ'CTC = change on Cerebral Tissular Concentration; Δ'CMR = Δ'CU − Δ'CTC = change on Cerebral Metabolic Rate; Δ'CMZR$_{NH_4^+}$ = change on Cerebral Metabolizing Rate for NH_4^+.

The symbol (●) indicates the statistical significance ($P < 0.05$) as compared with perfusion without *S*-adenosyl-L-methionine or adenosine: (*b*) and (*c*) versus (*a*)

Table 3. Actionof ammonium acetate (added to the cerebral perfusing blood: 2×10^{-4} M) on some parameters related to the glucidic metabolism and evaluated in cerebral tissue and by arterovenous differences across the brain*

Parameters		Unit of measurement	Control condition (*a*)	Perfusion with ammonium acetate after and during the infusion with: (*b*)	*S*-Adenosyl-L-methionine (4×10^{-4} M) (*c*)	Adenosine (4×10^{-4} M) (*d*)
From arterovenous differences across the brain	Cerebral blood flow	ml min^{-1} g^{-1}	0.494 ± 0.011	0.492 ± 0.010	0.477 ± 0.012	0.485 ± 0.015
	Glucose uptake	μmoles g^{-1} min^{-1}	0.285 ± 0.008	0.322 ± 0.006●	0.319 ± 0.014●	0.325 ± 0.007●
	Lactate release		0.038 ± 0.002	0.075 ± 0.005●	0.074 ± 0.005●	0.081 ± 0.005●
	Oxygen uptake		1.64 ± 0.07	1.73 ± 0.04	1.76 ± 0.02	1.75 ± 0.03
Concentration in brain	Glycogen	μmoles g^{-1}	3.48 ± 0.16	2.12 ± 0.03●	2.44 ± 0.07●▲	2.34 ± 0.06●
	Glucose		1.44 ± 0.15	2.66 ± 0.07●	2.28 ± 0.06●▲	2.48 ± 0.06●
	Pyruvate		0.100 ± 0.008	0.104 ± 0.004	0.102 ± 0.003	0.103 ± 0.002
	Lactate		1.64 ± 0.15	2.23 ± 0.07●	1.96 ± 0.04●▲	2.06 ± 0.04●
	ATP	μmoles g^{-1}	2.21 ± 0.04	2.26 ± 0.05	2.16 ± 0.07	2.20 ± 0.07
	ADP		0.48 ± 0.02	0.45 ± 0.01	0.52 ± 0.03	0.43 ± 0.02
	AMP		0.06 ± 0.002	0.09 ± 0.003	0.05 ± 0.003	0.08 ± 0.002
	Creatine phosphate		4.82 ± 0.12	4.40 ± 0.36	4.76 ± 0.20	4.59 ± 0.23

* The values are means (± standard errors) of six preparations. The symbol (●) indicates the statistical significance as compared with control condition: (*b*), (*c*) and (*d*) versus (*a*). The symbol (▲) in the hyperammonetmic animals indicates the statistical significance as compared with dogs not infused by *S*-adenosyl-L-methionine or adenosine: (*c*) and (*d*) versus (*b*). Level of statistical significance: $P < 0.05$.

Table 4. Changes (Δ') induced by ammonium acetate (added to the cerebral perfusing blood: 2×10^{-4} M) on some parameters related to the glucidic metabolism of the brain as compared with control condition*

Cerebral infusion with	Metabolite	Δ'CU	Δ'CTC	Δ'CMR	Δ'CAnMR$_{glucose}$	Δ'CAeMR$_{glucose}$	Δ'(ΔG')	
		nmoles g^{-1} min^{-1}					mcal g^{-1} min^{-1}	%
	Glucose	+37	+61	−24				
	Glycogen		−68	+68		+11	−7.55	83
	Lactate	−37	+29	−66	+33		−1.55	17
(*a*)	Total						−9.10	100
S-Adenosyl-L-methionine (4×10^{-4} M)	Glucose	+34	+42●	−8●				
	Glycogen		−52●	+52●		+18●	−12.35●	91●
	Lactate	−36	+16●	−52●	+26●		−1.22●	9●
(*b*)	Total						−13.57●	100
Adenosine (4×10^{-4} M)	Glucose	+40	+52	−12●				
	Glycogen		−57	+57		+13	−8.92	86
	Lactate	−43	+21	−64	+32		−1.50	14
(*c*)	Total						−10.42	100

* The calculated parameters (see also *Materials and Methods*) are:
Δ'CU = change on Cerebral Uptake; Δ'CTC = change on Cerebral Tissular Concentration; Δ'CMR = Δ'CU − Δ'CTC = change on Cerebral Metabolic Rate; Δ'CAnMR$_{glucose}$ = −0.5 (Δ'CMR$_{lactate}$) = change on Cerebral Anaerobic Metabolic Rate for glucose; Δ'CAeMR$_{glucose}$ = Δ'CMR$_{glucose}$ + Δ'CMR$_{glycogen}$ − Δ'CAnMR$_{glucose}$ = change on Cerebral Aerobic Metabolic Rate for glucose; Δ'(ΔG°) = change on Gibbs free energy.
The symbol (●) indicates the statistical significance as compared with perfusion without *S*-adenosyl-L-methionine or adenosine: (*b*) and (*c*) versus (*a*). Level of statistical significance: $P < 0.05$.

cating power of brain tissue, whereas the perfusion with *S*-adenosyl-L-methionine (0.4 mM) increases the metabolization of NH_4^+. This is caused by a higher ammonium-detoxicating power of glutamine matched by a decrease in that of aspartate. The ratio between ammonium-detoxicating power and ammonia metabolic rate (the *Metabolizing Power Index* for NH_4^+) is always very close to 1. This index, in fact, has a value of 0.94 in hyperammonemia, and values of 0.98 or 0.94 in the hyperammonemia developing during perfusion with *S*-adenosyl-L-methionine or adenosine, respectively.

Ammonium acetate induces a significant increase both in glucose uptake and in the formation of lactate, while O_2 uptake is not significantly increased (Table 3). At cerebral level, an increase in the concentrations of glucose and lactate can be observed, while a glycogen depletion occurs and tissular levels of pyruvate remain unchanged. There were no NH_4^+-induced modifications in the concentrations of ATP, ADP, AMP and creatine phosphate, indicating no change in the phosphorylation state of the adenine nucleotides in the brain. Computations quoted in Table 4 show that hyperammonemia induces a remarkable increase in the Gibbs free energy. The intervention of *S*-adenosyl-L-methionine increases the Gibbs free energy change by displacing the increased metabolism of glucides towards aerobic degradation.

Table 5. Action of ammonium acetate (added to cerebral perfusing blood: 2×10^{-4} M) on some parameters related to the *resting transmembrane potential* and evaluated both in arterial blood and in cerebral tissue.*

Parameters	Unit of measurement	Control conditions	Perfusion with ammonium acetate after and during the infusion with — (*a*)	*S*-Adenosyl-L-methionine (4×10^{-4} M) (*b*)	Adenosine (4×10^{-4} M) (*c*)
K^+ arterial		4.12 ± 0.16	4.71 ± 0.03●	4.88 ± 0.04●	4.77 ± 0.03●
K^+ cerebral		120.1 ± 1.5	116.5 ± 0.5	114.2 ± 0.4	116.4 ± 0.4
NH_4^+ arterial	μmoles g^{-1}	0.022 ± 0.003	0.214 ± 0.007●	0.226 ± 0.004●	0.221 ± 0.004●
NH_4^+ cerebral		0.23 ± 0.04	0.42 ± 0.01●	0.44 ± 0.02●	0.39 ± 0.03●
Calculated resting transmembrane potential	mV	−90.88 ± 1.26	−86.20 ± 0.63●	−84.63 ± 0.28●	−85.79 ± 0.17●

The resting transmembrane potential was calculated as:

$$-\frac{RT}{F}\ln\left(\frac{[K^+]_{int} + \alpha[NH_4^+]_{int}}{[K^+]_{ext} + \alpha[NH_4^+]_{ext}}\right),$$

where the subscripts *int* and *ext* refer to intracellular (cerebral) and extracellular (arterial) concentration respectively, and α is the permeability of NH_4^+ relative to K^+.

* The values are means (± standard errors) of six preparations of beagle dog. The symbol (●) indicates the statistical significance as compared with control condition. The symbol (▲) in hyperammonemic dogs indicates the statistical significance as compared with animal not infused by *S*-adenosyl-L-methionine or adenosine: (*c*) and (*a*) versus (*b*). Level of statistical significance: $P < 0.05$.

Table 6. Energy required for glutamine production

Pretreatment and treatment with	Glutamine● production	Energy required	Gibbs free●● energy	Excess of free energy
	nmoles g^{-1} min^{-1}		mcal g^{-1} min^{-1}	
S-Adenosyl-L-methionine	43	0.84	9.10	8.26
	54	1.05	13.57	12.52
Adenosine	48	0.94	10.42	9.48

● Data from Table 2.
●● Data from Table 4.

Hyperammonemia makes the *resting transmembrane potential*, indirectly obtained by applying the Nernst equation, less negative (Table 5). The extent of this event is slightly increased by the cerebral perfusion with S-adenosyl-L-methionine and, to a lesser degree, with adenosine.

DISCUSSION

As proposed also on the basis of previous studies, the increase in glutamine synthesis represents the major mechanism in *ammonia detoxication*. In fact, in cat brain ^{15}N from [^{15}N]ammonium acetate was found to be preferentially incorporated into the amino group of glutamine, as compared with the amino group of glutamate [34]. Furthermore, in the presence of an increased level of ammonia, in the brain cortex of the cat the specific activity of tissue $^{14}CO_2$ for glutamine increased without a corresponding decrease in specific activities for glutamate or aspartate [35]. In agreement with the results obtained in rat brain [33] frozen within 1 sec by the brain-freezer [36], in the present study the NH_4^+-induced alteration in the glutamate–ammonia system of the motor area of dog brain cortex suggests (Table 2) that : (1) the cerebral metabolizing rate for NH_4^+ was increased; (2) much NH_4^+ was incorporated into glutamine and a lesser amount into alanine; (3) the NH_4^+-induced change in the cerebral metabolic rate of glutamate, oxaloacetate, pyruvate and α-oxoglutarate was negligible; (4) the cerebral metabolic rate of aspartate was decreased; (5) pretreatment with S-adenosyl-L-methionine produced an increase in the NH_4^+-induced synthesis of extra glutamine. Such a result confirms *in vivo* that the glutamate–ammonia system forms an integral whole [37] in which fluctuations in the value of one component, such as ammonia, are compensated by corresponding fluctuations in the values of other components. At any rate, the glutamate ammonia system plays a regulatory role in the cerebral process of NH_4^+ detoxication. Indeed, the metabolizing power index for NH_4^+ respectively showed a value of 0.94 during the hyperammonemia syndrome, and values of 0.98 or 0.94 during the hyperammonemia syndrome elicited in the course of the brain perfusion with S-adenosyl-L-methionine or adenosine.

The *interference of S-adenosyl-L-methionine* on NH_4^+ detoxication is probably related to the formation of L-cysteine and its subsequent condensation with α-oxoglutarate to form mercaptopyruvate and glutamate, which may then be further converted to glutamine. In fact S-adenosyl-L-methionine provides the methyl groups to the acceptors (38) with the formation of S-adenosyl-L-homocysteine which is cleaved to L-homocysteine and adenosine; L-homocysteine may then undergo remethylation to L-methionine or enter the transsulfuration pathway with the formation of L-cysteine [39, 40]. Although in the present research no attention was directed to the problem of the blood–brain barrier penetration of the substance, it should be noted that the 100 mg/kg i.v. injection of S-adenosyl-L-methionine in rats induces the 100 per cent increase of the substance in the brain within 5 min. S-adenosyl-L-methionine i.v. injected to the rat, rabbit and man exhibits a $t_{50\%}$ of about 40 min for rats and rabbits, and of 80 min for man [42, 43]. We suggest that the interference of S-adenosyl-L-methionine on cerebral NH_4^+ detoxication is probably related to its increased cerebral concentration, which in our experimental model was induced by the direct infusion of the substance into cerebral circulation.

As for the problem of the change induced by NH_4^+ on *cerebral energetics*, the view that NH_4^+ interferes with "high-energy bond" phosphate seems unlikely. In fact, in agreement with the results obtained in rat brain [33, 41], in the present research adenine nucleotides concentration was unchanged, indicating no decrease in the phosphorylation state of the brain during acute hyperammonemia. On the contrary, we observed a significant increase of Gibbs free energy (Table 4) that can be utilized by a process which occurred as a consequence of increased [NH_4^+], such as: incorporation of NH_4^+ into glutamine, increase of amino acids transport to the brain, or ionic effects of ammonia.

As for NH_4^+ incorporation into glutamine, in the amidation reaction catalyzed by *glutamine synthase* the free standard energy expenditure is 7 kcal mol^{-1}, depending on the transformation of ATP to ADP. However, during glucose degradation, energy is transduced into ATP with a yield of 36 per cent. Therefore, in order to perform the amidation reaction a gross energy expenditure of approximately 19.5 kcal $moles^{-1}$ should be computed. By taking into account the values of glutamine production, the gross energy required for this production, under the various experimental conditions, can be calculated. The data summarized in Table 6 clearly show that the amount of energy required for the amidation of glutamate to glutamine is much lower than the change of Gibbs free energy caused by the hyperammonemia syndrome.

As for the increase in the amino acid transport to the cerebral tissue, it should be noted that the NH_4^+-induced changes in the uptake of glutamate, glutamine and alanine (Table 2) across the brain are negligible. This observation can not lead to any conclusive

data, because other amino acids may be carried to the brain and converted by the cerebral tissue. In fact in the blood–brain barrier there is one carrier for neutral amino acids and one for basic amino acids that can, for their transported substances, compete successfully against even strong hydrogen bonding to plasma water [44].

An ammonia-induced decrease in the resting transmembrane potential of about 4.5 to 6.0 mV was calculated from the Nernst equation in our various experimental conditions at an NH_4^+ blood level of 0.2 mM. In brain of ammonium acetate injected rats, with an NH_4^+ arterial blood of 1.7 mM (33), a decrease in the resting transmembrane potential of about 15 mV was indirectly calculated. This agrees with actual direct measurements made in cat motor neurons and squid giant axons, indicating that NH_4^+ decreases the resting transmembrane potential, therefore bringing the potential closer to the threshold for firing [1–3]; *S*-adenosyl-L-methionine seems to magnify this decrease. In cerebral tissue, NH_4^+ may exchange with K^+ [45], a K^+ efflux from brain being induced. In fact, after 20 min period of constant 0.2 mM ammonium acetate infusion, plasma $[K^+]$ rose from 4.1 to 4.7 μmoles g^{-1}, while cerebral $[K^+]$ decreased from 120.1 to 116.5 μmoles g^{-1}. According to the result obtained in rat brain (33), it may be suggested that the increased $[NH_4^+]$ and the resulting rise in $[K^+]$ may account, at least in part, for the changed metabolic rate. This action could be brought about by the activation of Na^+-, K^+-stimulated adenosine triphosphatase activity [46]. The stimulation of brain oxidative metabolic rate is proportionately related to plasma $[NH_4^+]$. In fact, in rat with 1.74 mM plasma $[NH_4^+]$, the brain oxygen uptake increased by 27.2 per cent [33], while in the present experiment in beagle dog with 0.2 mM plasma $[NH_4^+]$, the brain oxygen uptake increased only by 5.5 per cent. Glucose, glycogen and lactate cerebral utilization or production increased quite proportionately in the two quoted experimental conditions, indicating the important role of NH_4^+ on the cerebral metabolic and detoxicating processes, involving also the ionic dynamic state.

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