

Choline-deprivation alters crucial brain enzyme activities in a rat model of diabetic encephalopathy

Charis Liapi · Argyro Kyriakaki · Apostolos Zarros · Panagiota Galanopoulou · Hussam Al-Humadi · Ismene Dontas · Konstantinos Voumvourakis · Stylianos Tsakiris

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Abstract Diabetic encephalopathy describes the moderate cognitive deficits, neurophysiological and structural central nervous system changes associated with untreated diabetes. It involves neurotoxic effects such as the generation of oxidative stress, the enhanced formation of advanced glycation end-products, as well as the disturbance of calcium homeostasis. Due to the direct connection of choline (Ch) with acetylcholine availability and signal transduction, a background of Ch-deficiency might be unfavorable for the pathology and subsequently for the treatment of several metabolic brain diseases, including that of diabetic encephalopathy. The aim of this study was to shed more light on the effects of adult-onset streptozotocin (STZ)-induced diabetes and/or Ch-deprivation on the activities of acetylcholinesterase (AChE) and two important

adenosinetriphosphatases, namely Na^+, K^+ -ATPase and Mg^{2+} -ATPase. Male adult Wistar rats were divided into four main groups, as follows: control (C), diabetic (D), Ch-deprived (CD), and Ch-deprived diabetic (D+CD). Deprivation of Ch was provoked through the administration of Ch-deficient diet. Both the induction of diabetes and the beginning of dietary-mediated provoking of Ch-deprivation occurred at the same day, and rats were killed by decapitation after 30 days (1 month; groups C1, D1, CD1 and D1+CD1) and 60 days (2 months; groups C2, D2, CD2 and D2+CD2, respectively). The adult rat brain AChE activity was found to be significantly increased by both diabetes (+10%, $p < 0.001$ and +11%, $p < 0.01$) and Ch-deprivation (+19%, $p < 0.001$ and +14%, $p < 0.001$) when compared to the control group by the end of the first (C1) and the second month (C2), respectively. However, the Ch-deprived diabetic rats' brain AChE activity was significantly altered only after a 60-day period of exposure, resulting in a +27% increase (D2+CD2 vs. C2, $p < 0.001$). Although the only significant change recorded in the brain Na^+, K^+ -ATPase activity after the end of the first month is attributed to Ch-deprivation (+21%, $p < 0.05$, CD1 vs. C1), all groups of the second month exhibited a statistically significant decrease in brain Na^+, K^+ -ATPase activity (−24%, $p < 0.01$, D2 vs. C2; −21%, $p < 0.01$, CD2 vs. C2; −22%, $p < 0.01$, D2+CD2 vs. C2). As concerns Mg^{2+} -ATPase, the enzyme's activity demonstrates no significant changes, with the sole exception of the D2+CD2 group (+21%, $p < 0.05$, D2+CD2 vs. C2). In addition, statistically significant time-dependent changes concerning the brain Mg^{2+} -ATPase activity were recorded within the diabetic ($p < 0.05$, D2 vs. D1) and the Ch-deprived ($p < 0.05$, CD2 vs. CD1) rat groups. Our data indicate that Ch-deprivation seems to be an undesirable background for the above-mentioned enzymatic activities under untreated diabetes, in a time-evolving way. Further studies on the issue should focus on a

C. Liapi · A. Kyriakaki · A. Zarros · P. Galanopoulou · H. Al-Humadi
Department of Pharmacology, Medical School,
National & Kapodistrian University of Athens,
Athens, Greece

A. Zarros · S. Tsakiris (✉)
Department of Physiology, Medical School,
National & Kapodistrian University of Athens,
PO Box 65257, 15401 Athens, Greece
e-mail: stsakir@med.uoa.gr

I. Dontas
Laboratory of Experimental Surgery & Surgical Research,
Medical School,
National & Kapodistrian University of Athens,
Athens, Greece

K. Voumvourakis
2nd Department of Neurology, Attikon University Hospital,
Medical School,
National & Kapodistrian University of Athens,
Athens, Greece

region-specific reevaluation of these crucial enzymes' activities as well as on the possible oxidative mechanisms involved.

Keywords Rat brain · Choline-deprivation · Diabetes · Streptozotocin · Acetylcholinesterase · Na⁺,K⁺-ATPase · Mg²⁺-ATPase

Introduction

Untreated diabetes mellitus is known to cause a variety of complications such as blindness, neurological and vascular complications, cerebrovascular disease and even premature death (Cohen et al. 2007; Rolo and Palmeira 2006). Untreated diabetes mellitus is also associated with moderate cognitive deficits, neurophysiological and structural central nervous system (CNS) changes (Alvarez et al. 2009); a condition that may be referred to as “diabetic encephalopathy” (Brands et al. 2003; Mijnhout et al. 2006). Diabetic encephalopathy involves neurotoxic effects such as the generation of oxidative stress (Kumar and Menon 1993), the enhanced formation of advanced glycation end-products (Ryle et al. 1997), as well as the disturbance of calcium (Ca²⁺) homeostasis (Levy et al. 1994).

Choline (Ch) is an important nutrient that is involved in many physiological functions (Zeisel 1981). Apart from being a structural component of acetylcholine (ACh), Ch plays a critical role in generating second messengers for cell membrane signal transduction (by being a component of certain phospholipids) (Canty and Zeisel 1994). Deprivation of Ch may lead to memory and growth disorders (Zeisel 2005; Liapi et al. 2007), hepatocellular modifications (Konstandi et al. 2009) and/or even hepatic tumorigenesis (Davis and Uthus 2004). Deficiency of Ch might be a frequent problem in clinical settings, since it can accompany various common pathological (alcoholism and malnutrition) or physiological states (pregnancy and lactation). Due to its direct connection with ACh availability and signal transduction, a background of Ch-deficiency might be unfavorable for the pathology and subsequently for the treatment of several metabolic brain diseases, including that of diabetic encephalopathy. In fact, diabetes itself is known to lower the brain uptake index (BUI) of Ch by disrupting the blood-brain barrier (BBB) Ch transport (Mooradian 1987).

The aim of this study was to shed more light on the effects of adult-onset streptozotocin (STZ)-induced diabetes and/or Ch-deprivation on the activities of acetylcholinesterase (AChE; a crucial membrane-bound enzyme involved in cholinergic neurotransmission) (Kouniniotou-Krontiri and Tsakiris 1989) and two important adenosinetriphosphatases, namely Na⁺,K⁺-ATPase (an enzyme implicated in neuronal excitability, metabolic energy production, as well as in the uptake and release of catecholamines, serotonin

and glutamate) (Bogdanski et al. 1968; Hernandez 1987; Lees et al. 1990; Mata et al. 1980; Sastry and Phillis 1977; Swann 1984) and Mg²⁺-ATPase (an enzyme functioning in order to maintain high brain intracellular Mg²⁺, thus possibly controlling the rate of protein synthesis and cell growth) (Sanui and Rubin 1982). Experiments were designed and conducted so as to provide a view of possible time-dependent alterations in the activities of the above mentioned crucial adult rat brain enzymes.

Materials and methods

Animals

Male adult albino Wistar rats (4 months old, weighting 240±26 g) were used in all experiments. The rats were purchased by the National Center of Scientific Research “Demokritos” (Agia Paraskevi, Athens, Greece) and were housed four in a cage, at a constant room temperature (22±1°C) under a 12-h light : 12-h dark (light 08:00–20:00 h) cycle. Food and water were provided ad libitum. Animals were cared for in accordance with the principles for the care, use and protection of experimental animals as set by the European Economic Community (EEC) Council Directive 86/609/EEC (EEC Council 1986) and aligned according to the Recommendation 2007/526/EU.

Induction of diabetes and Ch-deprivation

Rats were divided into four main groups (*n*=12 at each group), as follows: control (C), diabetic (D), Ch-deprived (CD), and Ch-deprived diabetic (D+CD). Diabetes was induced with a single intraperitoneal (ip) injection of STZ (50 mg/kg of body weight, diluted in a 0.1 mol/L citrate solution, pH 4.5) (Bilginoglu et al. 2007; Zarros et al. 2009), while the rats belonging to groups C and CD were injected with the citrate buffer. Deprivation of Ch was provoked through the administration of Ch-deficient diet (CDD). Both the induction of diabetes and the beginning of dietary-mediated provoking of Ch-deprivation occurred at the same day, and rats were killed after 30 days (1 month; groups C1, D1, CD1 and D1+CD1) and 60 days (2 months; groups C2, D2, CD2 and D2+CD2, respectively). Two rats (one of the D2 and one of the D2+CD2 group) were not efficiently made diabetic and were excluded. The required CDD was obtained from Mucedola (Italy),¹ while all

¹ Ingredients of the CDD: sucrose, coconut oil, starch wheat, dextrine, extracted peanut meal, soy protein, corn oil, dicalcium phosphate, cellulose, potassium citrate, sodium chloride, magnesium oxide, L-cystine, vitamin A, vitamin D3, vitamin E (alpha-tocopherol), copper (copper sulfate pentahydrate), selenium (sodium selenite). Analysis: protein (12%), fat (16%), fiber (2%), ash (3.5%).

reagents used were of the highest quality grade available and were purchased from Sigma Chemicals (Germany).

Tissue preparation

The animals were sacrificed by decapitation and their whole brains were rapidly removed (and stored at -80°C until use). The tissue was homogenized in 10 vol ice-cold ($0-4^{\circ}\text{C}$) medium containing 50 mM Tris (hydroxymethyl) aminomethane-HCl (Tris-HCl), pH 7.4 and 300 mM sucrose, using an ice-chilled glass homogenizing vessel at 900 rpm (4–5 strokes). Then, the homogenate was centrifuged at $1,000 \times g$ for 10 min to remove nuclei and debris (Tsakiris 2001; Tsakiris et al. 2000). In the resulting supernatant, the protein content was determined according to the method of Lowry et al. (1951) and then the enzyme activities were measured.

Determination of brain AChE activity

AChE activity was determined by following the hydrolysis of acetylthiocholine according to the method of Ellman et al. (1961), as previously described by Tsakiris (2001). The incubation mixture (1 ml) contained 50 mM Tris-HCl, pH 8, 240 mM sucrose and 120 mM NaCl. The protein concentration of the incubation mixture was 80–100 $\mu\text{g}/\text{ml}$. The reaction was initiated after addition of 0.03 ml of 5,5'-dithionitrobenzoic acid (DTNB) and 0.05 ml of acetylthiocholine iodide, which was used as substrate. The final concentration of DTNB and substrate were 0.125 and 0.5 mM, respectively. The reaction followed spectrophotometrically by the increase of absorbance ($\Delta\overline{OD}$) at 412 nm.

Determination of Na^+, K^+ -ATPase and Mg^{2+} -ATPase activities

(Na^+, K^+)-ATPase activity was calculated from the difference between total ATPase activity ($\text{Na}^+, \text{K}^+, \text{Mg}^{2+}$ -dependent ATPase) and Mg^{2+} -dependent ATPase activity. Total ATPase activity was assayed in an incubation medium consisting of 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 20 mM KCl, 4 mM MgCl_2 , 240 mM sucrose, 1 mM ethylenediamine tetraacetic acid K_2 -salt (K^+ -EDTA), 3 mM disodium ATP and 80–100 μg protein of the homogenate in a final volume of 1 ml. Ouabain (1 mM) was added in order to determine the activity of Mg^{2+} -ATPase. The reaction was started by adding ATP and stopped after an incubation period of 20 min by addition of 2 ml mixture of 1% lubrol and 1% ammonium molybdate in 0.9 M H_2SO_4 (Bowler and Tirri 1974; Tsakiris 2001). The yellow colour which developed was read at 390 nm.

Statistical analysis

The data were analyzed by Student's *t*-test followed by Bonferroni post-hoc test when needed. All analyses were performed by SPSS 16.1 Statistical Package for Windows Software, while *p* values of <0.05 were considered statistically significant.

Results

The adult rat brain AChE activity (Table 1) was found to be significantly increased by both diabetes (+10%, $p<0.001$ and +11%, $p<0.01$) and Ch-deprivation (+19%, $p<0.001$ and +14%, $p<0.001$) when compared to the control group by the end of the first (C1) and the second month (C2), respectively. However, the Ch-deprived diabetic rats' brain AChE activity was significantly altered only after a 60-day period of exposure, resulting in a +27% increase (D2+CD2 vs. C2, $p<0.001$; Table 1). Figure 1 provides an overview of the time-dependent changes occurring among the experimental groups' brain AChE activity, indicating significant alterations as concerns this important enzymatic parameter under both Ch-deprivation ($p<0.01$, CD2 vs. CD1) and its co-existence with diabetes ($p<0.001$, D2+CD2 vs. D1+CD1).

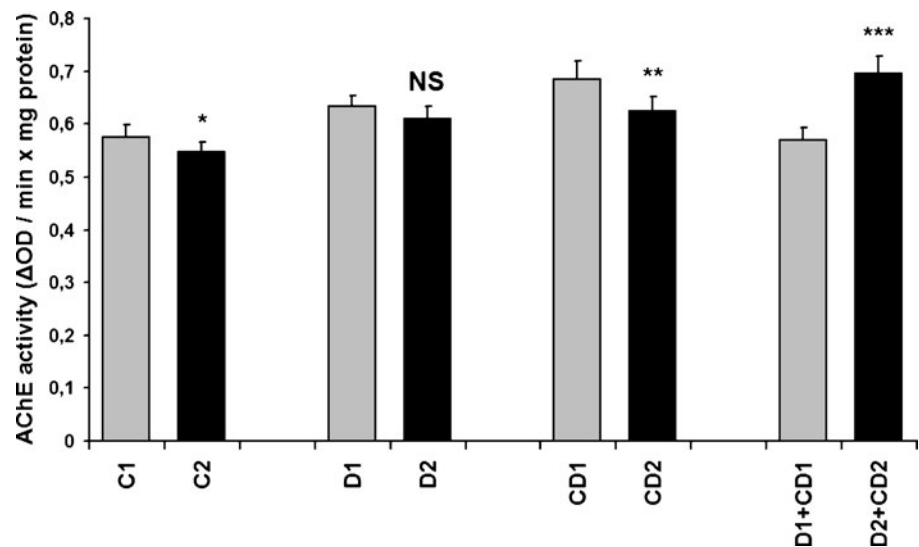
The effects of adult-onset Ch-deprivation and/or diabetes on the rat brain Na^+, K^+ -ATPase activity are presented in Table 2. Although the only significant change recorded in the brain Na^+, K^+ -ATPase activity at the end of the first month is attributed to Ch-deprivation (+21%, $p<0.05$, CD1

Table 1 Effects of streptozotocin-induced diabetes and/or choline-deprivation on the brain acetylcholinesterase (AChE) activity of adult rats

Group	AChE ($\Delta\overline{OD}/\text{min} \times \text{mg protein}$)		
	mean \pm SD	vs. C1	vs. D1
C1 ($n=6$)	0.575 \pm 0.023 ^a	–	–
D1 ($n=6$)	0.634 \pm 0.019 ^b	(+10%)	–
CD1 ($n=6$)	0.685 \pm 0.034 ^c	(+19%)	–
D1+CD1 ($n=6$)	0.570 \pm 0.023 ^d	(–1%)	(–10%)
	mean \pm SD	vs. C2	vs. D2
C2 ($n=6$)	0.548 \pm 0.018 ^e	–	–
D2 ($n=5$)	0.609 \pm 0.025 ^f	(+11%)	–
CD2 ($n=6$)	0.625 \pm 0.027 ^g	(+14%)	–
D2+CD2 ($n=5$)	0.696 \pm 0.033 ^h	(+27%)	(+14%)

For the meaning of Group abbreviations, see “Materials and methods”. Each value indicates the mean \pm SD of five or six independent experiments (five or six rats per group). The average of each experiment arose from three evaluations of the homogenized brain of each animal. Statistics: a/b, a/c, b/d, e/g, e/h: $p<0.001$; e/f, f/h: $p<0.01$; a/d: $p>0.05$

Fig. 1 Time-dependent effects of streptozotocin-induced diabetes and/or choline-deprivation on the brain acetylcholinesterase (AChE) activity of adult rats. For the meaning of Group abbreviations, see “Materials and methods”. Each value indicates the mean \pm SD of five or six independent experiments (five or six rats per group). The average of each experiment arose from three evaluations of the homogenized brain of each animal. Statistical significance is indicated towards the respective first month group. ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; NS: $p > 0.05$



vs. C1), the second month findings are impressive: all groups exhibit a statistically significant decrease in brain Na^+, K^+ -ATPase activity (-24% , $p < 0.01$, D2 vs. C2; -21% , $p < 0.01$, CD2 vs. C2; -22% , $p < 0.01$, D2+CD2 vs. C2; Table 2). These time-dependent changes are overviewed in Fig. 2, where the main finding is the significant decrease in the Na^+, K^+ -ATPase activity ($p < 0.001$, CD2 vs. CD1), estimated at about 29%.

As concerns Mg^{2+} -ATPase, Table 3 presents our findings on its activity in rat brain homogenates after 1 and 2 months of adult-onset Ch-deprivation and/or diabetes. The enzyme's activity demonstrates no significant changes, with the sole exception of the D2+CD2 group ($+21\%$, $p < 0.05$, D2+CD2 vs. C2). In addition, statistically significant

time-dependent changes concerning the brain Mg^{2+} -ATPase activity were recorded within the diabetic ($p < 0.05$, D2 vs. D1) and the Ch-deprived ($p < 0.05$, CD2 vs. CD1) rat groups (Fig. 3).

Discussion

Our data shed light on the effects of two important metabolic states (that have been proved to seriously affect optimal brain functioning in multiple ways) on well-studied crucial rat brain enzyme activities, such as those of AChE, (Na^+, K^+)-ATPase and Mg^{2+} -ATPase. Adult-onset untreated diabetes is a known oxidative state (Biessels et al. 2002; Tahirovic et al. 2007; Zarros et al. 2009) that can provoke extensive neurochemical, structural and functional deficits (Brands et al. 2003; Chu et al. 1986; Wahba and Soliman 1988), while dietary-induced Ch-deprivation is a well-established experimental model for simulating multiple clinical states (Konstandi et al. 2009; Liapi et al. 2009b; Zeisel 1981, 2000) with significant neurotoxic consequences.

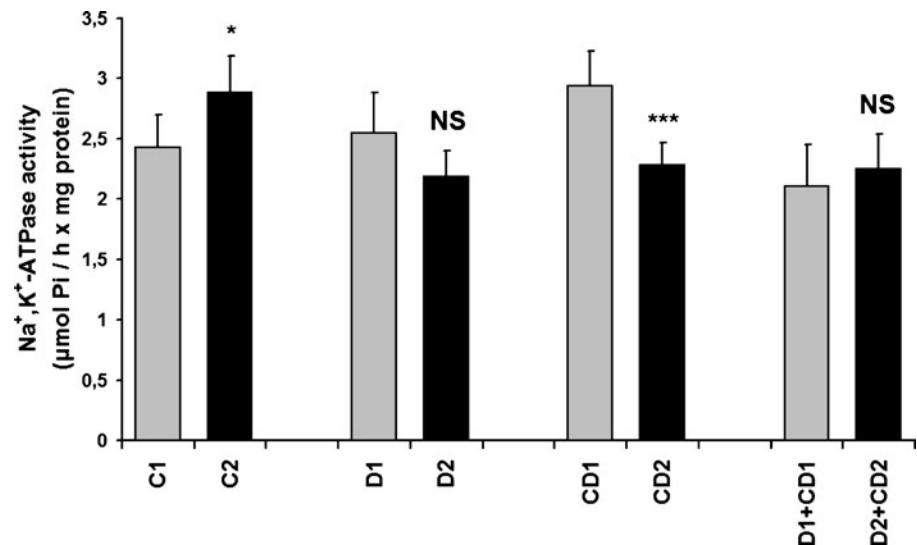
Our findings concerning the effect of adult-onset STZ-induced diabetes on the rat brain AChE activity are in accordance with those of our recently published study (Zarros et al. 2009) and also add a novel finding: AChE activity is not only significantly increased (vs the control state) after a 2-month period following the adult-onset induction of diabetes, but seems to be significantly increased at least since the end of the first month (Table 1, Fig. 1). Moreover, the previously reported (Zarros et al. 2009) effects of diabetes on the adult rat brain Na^+, K^+ -ATPase activity are also re-confirmed by this study. The significantly decreased Na^+, K^+ -ATPase activity (vs the control state) by the end of the second month following the induction of diabetes is a constant finding (Zarros et al.

Table 2 Effects of streptozotocin-induced diabetes and/or choline-deprivation on the brain Na^+, K^+ -ATPase activity of adult rats

Group	Na^+, K^+ -ATPase ($\mu\text{mol Pi/h} \times \text{mg protein}$)		
	mean \pm SD	vs. C1	vs. D1
C1 ($n=6$)	2.43 \pm 0.27 ^a	–	–
D1 ($n=6$)	2.55 \pm 0.33 ^b	(+5%)	–
CD1 ($n=6$)	2.94 \pm 0.29 ^c	(+21%)	–
D1+CD1 ($n=6$)	2.11 \pm 0.34 ^d	(–13%)	(–17%)
	mean \pm SD	vs. C2	vs. D2
C2 ($n=6$)	2.88 \pm 0.31 ^e	–	–
D2 ($n=5$)	2.19 \pm 0.21 ^f	(–24%)	–
CD2 ($n=6$)	2.28 \pm 0.19 ^g	(–21%)	–
D2+CD2 ($n=5$)	2.25 \pm 0.29 ^h	(–22%)	(+3%)

For the meaning of Group abbreviations, see “Materials and methods”. Each value indicates the mean \pm SD of five or six independent experiments (five or six rats per group). The average of each experiment arose from three evaluations of the homogenized brain of each animal. Statistics: e/f, e/g, e/h: $p < 0.01$; a/c, b/d: $p < 0.05$; a/b, a/d, f/h: $p > 0.05$

Fig. 2 Time-dependent effects of streptozotocin-induced diabetes and/or choline-deprivation on the brain Na^+, K^+ -ATPase activity of adult rats. For the meaning of Group abbreviations, see “Materials and methods”. Each value indicates the mean \pm SD of five or six independent experiments (five or six rats per group). The average of each experiment arose from three evaluations of the homogenized brain of each animal. Statistical significance is indicated towards the respective first month group. ***: $p < 0.001$; *: $p < 0.05$; NS: $p > 0.05$



2009; Table 2) that is in accordance with the literature (Franzon et al. 2005; Leong and Leung 1991). However, a novel finding emerges: Na^+, K^+ -ATPase is not significantly altered in the earlier period (at least as monitored by the end of the first month following the experimental induction of diabetes). This gradually-developing decrease in the rat brain Na^+, K^+ -ATPase activity (existent but non-statistically significant as measured among the D2 and D1 groups, Fig. 2) could be attributed to both the diabetic encephalopathy-linked brain mitochondrial dysfunction (Mastrocola et al. 2005) and the reported diabetes-induced purinergic signaling modification (Duarte et al. 2007), both correlated to decreased ATP availability. Moreover, this finding may

reflect a possible mean through which adult-onset untreated diabetes gradually and time-dependently affects neuronal excitability, metabolic energy production and certain systems of neurotransmission (Bogdanski et al. 1968; Hernandez 1987; Lees et al. 1990; Mata et al. 1980; Sastry and Phillis 1977; Swann 1984), thus contributing to the disease's neuropsychiatric phenotype.

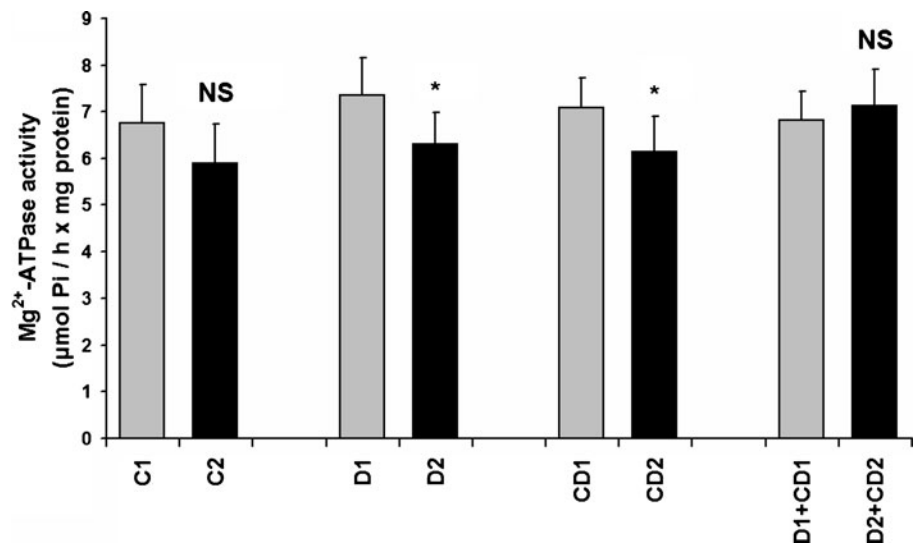
In the case of Ch-deprivation, a significant increase in rat brain AChE activity as a result of adult-onset dietary-induced Ch-deprivation is a finding we did not expect for the rat brain acquired at the end of the first month (CD1, Table 1): in a recently published study (Liapi et al. 2009a), we had recorded a non-significant increase in AChE activity (+9%, $n=5$, $p > 0.05$) over the same period of dietary Ch-deprivation. Although conducted on slightly older and heavier rats in larger groups, our current study does not establish a final verdict on the whole brain AChE behavior in adult-onset Ch-deprivation. Gestational Ch-deprivation has been reported to decrease the offspring brain AChE activity (Liapi et al. 2007), while prolonged Ch-deprivation (though gestation, lactation and an additional 3-week-period) has been shown to increase the offspring brain AChE activity (Liapi et al. 2008). Taking under consideration the fact that the administration of Ch is known to increase the brain ACh levels (either due to a precursor-induced enhancement of ACh biosynthesis or due to a central muscarinic response) (Cohen and Wurtman 1976; Kilbinger and Kruehl 1981), Ch-deprivation would be expected to cause a decrease in AChE activity. However, this was once more not observed. In fact, this significant increase in AChE activity (compared to the respective control state, C1) was also confirmed by the end of the second month of the current experiment (although facing a trend to be eliminated in means of absolute activity, see Fig. 1).

Table 3 Effects of streptozotocin-induced diabetes and/or choline-deprivation on the brain Mg^{2+} -ATPase activity of adult rats

Group	Mg^{2+} -ATPase ($\mu\text{mol Pi/h} \times \text{mg protein}$)		
	mean \pm SD	vs. C1	vs. D1
C1 ($n=6$)	6.75 \pm 0.83 ^a	–	–
D1 ($n=6$)	7.36 \pm 0.80 ^b	(+9%)	–
CD1 ($n=6$)	7.09 \pm 0.64 ^c	(+5%)	–
D1+CD1 ($n=6$)	6.82 \pm 0.61 ^d	(+1%)	(–7%)
	mean \pm SD	vs. C2	vs. D2
C2 ($n=6$)	5.89 \pm 0.84 ^e	–	–
D2 ($n=5$)	6.30 \pm 0.69 ^f	(+7%)	–
CD2 ($n=6$)	6.13 \pm 0.76 ^g	(+4%)	–
D2+CD2 ($n=5$)	7.13 \pm 0.77 ^h	(+21%)	(+13%)

For the meaning of Group abbreviations, see “Materials and methods”. Each value indicates the mean \pm SD of five or six independent experiments (five or six rats per group). The average of each experiment arose from three evaluations of the homogenized brain of each animal. Statistics: e/h: $p < 0.05$; a/b, a/c, a/d, b/d, e/f, e/g, f/h: $p > 0.05$

Fig. 3 Time-dependent effects of streptozotocin-induced diabetes and/or choline-deprivation on the brain Mg^{2+} -ATPase activity of adult rats. For the meaning of Group abbreviations, see “Materials and methods”. Each value indicates the mean \pm SD of five or six independent experiments (five or six rats per group). The average of each experiment arose from three evaluations of the homogenized brain of each animal. Statistical significance is indicated towards the respective first month group. *: $p < 0.05$; NS: $p > 0.05$



Our recent hypothesis (Liapi et al. 2009a) attributed the observed (at that study) rat brain AChE stability to the fact that the experimentally-induced Ch-deprivation was a 30-day adult-onset one, and thus, not long enough in order to deplete the adult rat body's Ch banks. This hypothesis cannot be excluded, but must be certainly modified now. The explanation may lay in our brain-region study (Liapi et al. 2009b) conducted on 2-month-long Ch-deprived adult rats. This study indicates a region specific behavior for AChE activity, ranging from statistically-significant increased recordings (cerebellum: +46%, $p < 0.001$; hippocampus: +28%, $p < 0.001$) to non-significant recordings (frontal cortex: -4%, $p > 0.05$; hypothalamus: +8%, $p > 0.05$; pons: +2%, $p > 0.05$). As also concluded by other studies on adult-onset hyper- and hypothyroidism (Carageorgiou et al. 2007a, b), the region-specific behavior of AChE activity under a metabolic state (such as Ch-deprivation in our case) might be the result of other neurotransmission-involving interactions that affect this cholinergic enzyme's activity in a region specific way towards the achievement of homeostatic reflexes that aim to maintain crucial functioning in crucial neuronal networks.

As concerns the activity of Na^+, K^+ -ATPase, this was found to be significantly increased by the end of the first and significantly decreased by the end of the second month by Ch-deprivation. Our finding (concerning the first month) is in accordance with our recently published ones (Liapi et al. 2009a), while one cannot easily explain the second month finding (Table 2, Fig. 2). Could this finding reflect certain modulations in metabolic energy production (Mata et al. 1980), necessary for the maintenance of efficient neuronal functioning under this advanced time-point of Ch-deprivation? Or is it a free-radical-related phenomenon that can be connected to the neurotoxic effects of oxidative

stress produced by the (at that time) established Ch-deficiency (Liapi et al. 2007)?

The induction of dietary Ch-deprivation in diabetic rats also exhibited peculiar and time-evolving phenomena on the examined enzymatic parameters: AChE activity was only significantly altered in the 2-month-exposure group (+27%, $p < 0.001$, D2+CD2 vs. C2, Table 1), while Na^+, K^+ -ATPase activity maintained a time-independent decrease compared to the respective controls (-13%, $p > 0.05$, D1+CD1 vs. C1; -22%, $p < 0.01$, D2+CD2 vs. C2; Table 2). Moreover, at the end of the second month, Mg^{2+} -ATPase activity exhibited the only statistically significant alteration recorded in this study (+21%, $p < 0.05$, D2+CD2 vs. C2), thus indicating a possible deregulation of the maintenance of high brain intracellular Mg^{2+} , and thus, possibly, a lack of control of the rate of protein synthesis and cell growth (Sanui and Rubin 1982).

In conclusion, our study sheds some light on the effects of adult-onset Ch-deprivation and/or STZ-induced diabetes on crucial rat brain enzyme activities, such as those of AChE, (Na^+, K^+)-ATPase and Mg^{2+} -ATPase. The induction of Ch-deprivation has an impact on the diabetes-induced effects on the above-mentioned enzymatic activities; an impact that evolves from a mild tendency to activity normalization (by the end of the first month of the study) to a maintenance or enhancement of the observed effects (by the end of the second month of the study). In fact, Ch-deprivation seems to be an undesirable background for the above-mentioned enzymatic activities under untreated diabetes, in a time-evolving way. Our study also concludes that whole brain AChE is either stimulated or maintained in normal activity levels during adult-onset Ch-deprivation in a: (a) region-specific, (b) time-dependent and (c) (possibly) multiply-regulated way. Further studies on the issue should

focus on a region-specific reevaluation of these crucial enzymes' activities as well as on the possible oxidative mechanisms involved.

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