Metab Brain Dis (2010) 25:211–217
DOI 10.1007/s11011-010-9198-6

ORIGINAL PAPER

Effect of ketogenic diet on nucleotide hydrolysis and hepatic enzymes in blood serum of rats in a lithium-pilocarpine-induced status epilepticus

Vanessa Gass da Silveira · Giana de Paula Cognato · Alexandre Pastoris Müller · Fabrício Figueiró · Carla Denise Bonan · Marcos L. Santos Perry · Ana Maria Oliveira Battastini

Received: 5 October 2009 / Accepted: 3 February 2010 / Published online: 5 May 2010

Abstract The ketogenic diet (KD) is a high-fat and low-carbohydrate diet, used for treating refractory epilepsy in children. We have previously shown alterations in nucleotidase activities from the central nervous system and blood serum of rats submitted to different models of epilepsy. In this study we investigated the effect of KD on nucleotidase activities in the blood serum, as well if KD has any influence in the activity of liver enzymes such as alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase activities in Wistar rats submitted to the lithium–pilocarpine model of epilepsy. At 21 days of age, rats received an injection of lithium chloride and, 18–19 h later, they received an injection of pilocarpine hydrochloride for status epilepticus induction. The results reported herein show that seizures induced by lithium–pilocarpine elicit a significant increase in ATP hydrolysis and alkaline phosphatase activity, as well as a decrease in ADP hydrolysis and aspartate aminotransferase activity. The KD is a rigorous regimen that can be associated with hepatic damage, as shown herein by the elevated activities of liver enzymes and 5′-nucleotidase in blood serum. Further studies are necessary to investigate the mechanism of inhibition of lithium on nucleotidases in blood serum.

Keywords Nucleotidases · Aminotransferases · Alkaline phosphatase · Status epilepticus · Ketogenic diet · Pilocarpine model

V. G. da Silveira · G. de Paula Cognato · A. P. Müller · F. Figueiró · M. L. S. Perry · A. M. O. Battastini (✉)
Departamento de Bioquímica,
Instituto de Ciências Básicas da Saúde,
Universidade Federal do Rio Grande do Sul,
Rua Ramiro Barcelos, 2600, Anexo,
90035-003 Porto Alegre, RS, Brazil
e-mail: abattastini@gmail.com

C. D. Bonan
Departamento de Biologia Celular e Molecular,
Faculdade de Biociências,
Pontifícia Universidade Católica do Rio Grande do Sul,
Avenida Ipiranga, 6681, Caixa Postal 1429,
90619-900 Porto Alegre, RS, Brazil

Introduction

The prospect that epilepsy might be controlled, at least partially, by nutritional modification is radical but highly appealing. The ketogenic diet (KD) is certainly the best-known dietary approach to epilepsy treatment (Stafstrom 2004). The KD was initially devised in 1921 to mimic the anticonvulsant effects of fasting, which were known to suppress seizures (Wilder 1921). Since both the KD and fasting have beneficial effects on epilepsy, it has been assumed that they share a common mechanism in alleviating seizures. In addition to ketosis, other changes associated with the ketogenic diet might affect seizure activity. For example, changes in energy metabolism, in lipid composition of cell membranes, in the level of brain water content, and in brain pH have all been suggested to play a role in seizure suppression (Schwartzkroin 1999; Janigro 1999).

Cholinomimetic agents are capable of inducing epileptic phenomena when applied either systemically or directly to the central nervous system (CNS). Convulsions induced by cholinergic stimulation produce a distinctive pattern of electroencephalographic, behavioral and pathological changes mimicking human epilepsy (Turski et al. 1989). Thus, seizures induced by cholinomimetic agents have been widely used as valuable models of experimental epilepsy

(Leite et al. 2002; Turski 2000; Turski et al. 1989). Interestingly, lithium pretreatment has been shown to potentiate the convulsant effect of cholinomimetics (Honchar et al. 1983; Morrisett et al. 1987a, b). Since this model reproduces most clinical, temporal and neuropathologic features of human temporal lobe epilepsy (Turski et al. 1989; Cavalheiro 1995; Dubé et al. 2001a, b; Rigoulot et al. 2004), we have chosen the lithium–pilocarpine model of epilepsy to induce the seizures in this work.

Extracellular nucleotides can be hydrolyzed by members of the E-NTPDase (ectonucleoside triphosphate diphosphohydrolase) family, E-NPP (ectonucleoside pyrophosphatase/phosphodiesterase) family and by alkaline phophatases (Zimmermann 2001). These ectonucleotidases, together with ecto-5′-nucleotidase, control the availability of ATP, ADP, AMP and adenosine for both nucleotide and nucleoside receptors and, consequently, the duration and extent of receptor activation (Chen and Guidotti 2001). Soluble nucleotidases have also been shown to be released from sympathetic nerves (Todorov et al. 1997) and as previously described by our laboratory, ATP and ADP can be hydrolyzed by the action of a soluble NTPDase and PDEase in rat blood serum (Oses et al. 2004).

The level of exogenous ATP may be increased in various inflammatory and shock conditions, mainly as a consequence of nucleotide release from platelets, endothelial and blood vessel cells (Dubyak 2000). This rise in exogenous ATP concentration is usually accompanied by concurrent secretion of various enzymes into the intercellular space (Yegutkin et al. 2000). Thus, the measurement of the rate of nucleotide hydrolysis in blood may serve as an auxiliary tool in the diagnosis of cellular damage in various pathophysiological conditions (Yegutkin 1997). Studies from our laboratory have evaluated the role of blood serum nucleotidase activities in the control of nucleotide levels during epilepsy or seizure events. Single PTZ injection led to significantly increased ATP, ADP and AMP hydrolysis in rat blood serum (Bruno et al. 2002) and in CSF (Oses et al. 2007). Likewise, animals subjected to PTZ-kindling demonstrated increased ATP, ADP and AMP hydrolysis in blood serum whereas phosphodiesterase activity was unchanged (Bruno et al. 2003). The fact that this increase can be measured in serum could mean that these enzymes might be promising plasma markers of seizures in epilepsy (Bruno et al. 2002). In addition, several reports have shown alterations in 5′-NT, alkaline phosphatase (ALP) and aminotransferases (ALT and AST) in different hepatobiliary disorders (Dixon and Purdom 1954; Hill and Sammons 1967; Song et al. 1969; Reichling and Kaplan 1988; Pagani and Panteghini 2001; Roberts 1930; Neuschwander-Tetri et al. 1996; Zhou et al. 1998). In fact, some of the enzymes studied herein can be increased in the presence of other pathological events, particularly hepatic disorders (Pagani and Panteghini 2001; Reichling and Kaplan 1988). Thus, to evaluate the possible liver damage, we measured the levels of the hepatic enzymes, alkaline ALP, ALT and AST in rat blood serum.

Considering the influence of KD and adenine nucleotides on the central and peripheral nervous system, the aim of this investigation was to study the effect of KD in ATP, ADP and AMP hydrolysis in the blood serum of Wistar rats, as well as its relationship with ALP and aminotransferases in a lithium-pilocarpine-induced SE.

Experimental procedure

Materials

Nucleotides (ATP, ADP, AMP), Hepes, Trizma base, EDTA, levamisole and pilocarpine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Diagnostic kits were manufactured by Labtest Diagnostic. All others reagents were of analytical grade.

Animals and lithium–pilocarpine model

Female Wistar rats (21 days-old) were maintained on a 12-h light/dark cycle in a ventilated room at 21°C with free access to food and water. All animal procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

At 21 days of age, 24 rats received an i.p. injection of lithium chloride (127 mg/kg) and 18–19 h later they received an i.p. injection of pilocarpine hydrochloride (60 mg/kg). After pilocarpine injection, all rats progressed to SE. The onset of SE was characterized by initial immobility and chewing followed by repetitive clonic activity of the trunk and limbs. The rats then developed repeated rearing with forelimb clonus and falling interspersed with immobility, chewing, and myoclonic jerks occurring singularly or in series (Cavalheiro et al. 1987; Zhao et al. 2004). Seizures featured SE last 4 h after the injection of pilocarpine. The SE was responsible for the death of two animals (both 2 h after the pilocapine administration) from the total of 24 that were submitted to lithium–pilocarpine model. A control group of 11 rats received an injection of the same amount of normal saline.

Diets

The ketogenic and control diets were prepared weekly and their composition is presented in the Table 1. The epileptic (n=22) and control rats (n=11), were fasted for 24 h prior to initiation of diets. The animals were divided into four groups: Control group (received an injection of saline and

Table 1 Composition of control and ketogenic diets

Control	g/100g	Ketogenic	g/100g
Lard	4.5	Lard	65.5
Soy oil	0.5	Soy oil	2.0
Soy protein[a]	25	Soy Protein[a]	25
Fiber	1.0	Fiber	1.0
Salt Mix[b]	4.0	Salt Mix[b]	4.0
Vitamin Mix[c]	1.0	Vitamin Mix[c]	1.0
DL-methionine[d]	0.3	DL-methionine[d]	0.3
Carbohydrates	63.7	Carbohydrates	1.2

[a] Soy protein purity 92% (BUNGE)

[b] Mineral mixture (Roche, São Paulo, Brazil), mg/100 g of ration: NaCl, 557; Kl, 3.2; KH2PO4, 1,556; MgSO4, 229; CaCO3, 1,526; FeSO4 _7H2O, 108; MnSO4 _ H2O, 16; ZnSO4 _ 7H2O, 2.2; CuSO4 _ 5H2O, 1.9; and CoCl2_6H2O, 0.09

[c] Vitamin mixture (Roche, São Paulo, Brazil), mg/100 g of ration: vitamin A, 4; vitamin D, 0.5; vitamin E, 10; menadione, 0.5; choline, 200; PABA 10; inositol 10 mg; niacin, 4; pantothenic acid, 4; riboflavin, 0.8; thiamin, 0.5; pyridoxine, 0.5; folic acid, 0.2; biotin, 0.04; and vitamin B-12, 0.003

[d] DL-methionine (Delaware, Porto Alegre, Brazil)

control diet; $n=5$); ketogenic group (received an injection of saline and ketogenic diet; $n=6$); lithium Pilocarpine group (submitted to lithium–pilocarpine model and received control diet; $n=11$); and lithium–pilocarpine-ketogenic group (submitted to lithium–pilocarpine model and received ketogenic diet; $n=11$). The rats had free access to food and water for 6 weeks after induction of SE (or not, in the case of control groups) and ketonemia was determining by a semiquantitative kit (Keto-Diabur-Test 5000) from Roche Diagnostics (Mannheim, Germany). After this period of treatment, the animals were killed by decapitation and blood was isolated for enzymatic analysis.

Isolation of blood serum fraction

Rats were decapitated and drainage from the cut surface. The blood samples were drawn and immediately centrifuged at 3,000 r.p.m. for 5 min at room temperature. The serum samples obtained were stored at −20°C until analysis

Measurement of ATP, ADP and AMP hydrolysis

ATP, ADP and AMP hydrolysis were performed using the method described previously by Oses et al. 2004. The reaction mixture containing ATP or ADP as substrate (3 mM) in 112.5 mM Tris–HCl, pH 8.0, was incubated with 1.0 mg of serum protein at 37°C for 40 min in a final volume of 0.2 mL. The reaction was stopped by the addition of 0.2 mL of 10% TCA. All samples were chilled on ice, centrifuged at 5,000 × g for 5 min and the amount of inorganic phosphate (Pi) liberated was measured by the malachite green method (Chan et al. 1986). AMP hydrolysis was determined in the same conditions for ATP and ADP, except that the substrate was AMP (3 mM) and at pH 7.5. To exclude a possible interference of non-specific phosphatases in nucleotide hydrolysis, we incubated the blood serum with levamisole, a specific inhibitor of alkaline phosphatase. For all enzyme assays, incubation time and protein concentration were chosen to ensure the linearity of the reaction. In order to correct non-enzymatic hydrolysis, we performed controls by adding the serum after the reaction was stopped with TCA. All samples were assayed in duplicate. Enzyme activities were expressed as nmol of Pi released per minute per milligram of protein.

Protein determination

Protein was measured by the Coomassie Blue method (Bradford 1976), using bovine serum albumin as standard.

Determinations of ALT, AST and ALP

Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Reitman and Frankel 1957) and alkaline phosphatase (ALP) (Roy 1970) in the serum were determined using commercial diagnostic kits, manufactured by Labtest Diagnostic, Brazil. Enzyme activities were expressed as U/L.

Statistical analysis

The data obtained are expressed as means±standard deviation of at least five animals. The results were analyzed statistically by two-way ANOVA followed by Bonferroni post hoc test (GraphPad Prism vs 5).

Results

Experiments using a KD with 20% protein, as described in the literature for the treatment of children with epilepsy, caused an important undernutrition in rats with a significant loss of weight and hair of rats (data not shown). Therefore, we chose a KD with 25% protein in order to avoid these side effects. With the treatment used here, the animals remained apparently healthy and a semi-quantitative test showed that the blood levels of ketone bodies in the serum of control rats were lower than 0.5 mmol/L, while animals fed on the ketogenic diet developed an increased level of ketosis (1–5 mmol/L).

Figure 1 shows the changes in the hydrolysis of nucleotides in the blood serum of female rats submitted to KD and to a lithium–pilocarpine-induced SE. The statistical analysis by two-way ANOVA showed that KD per se

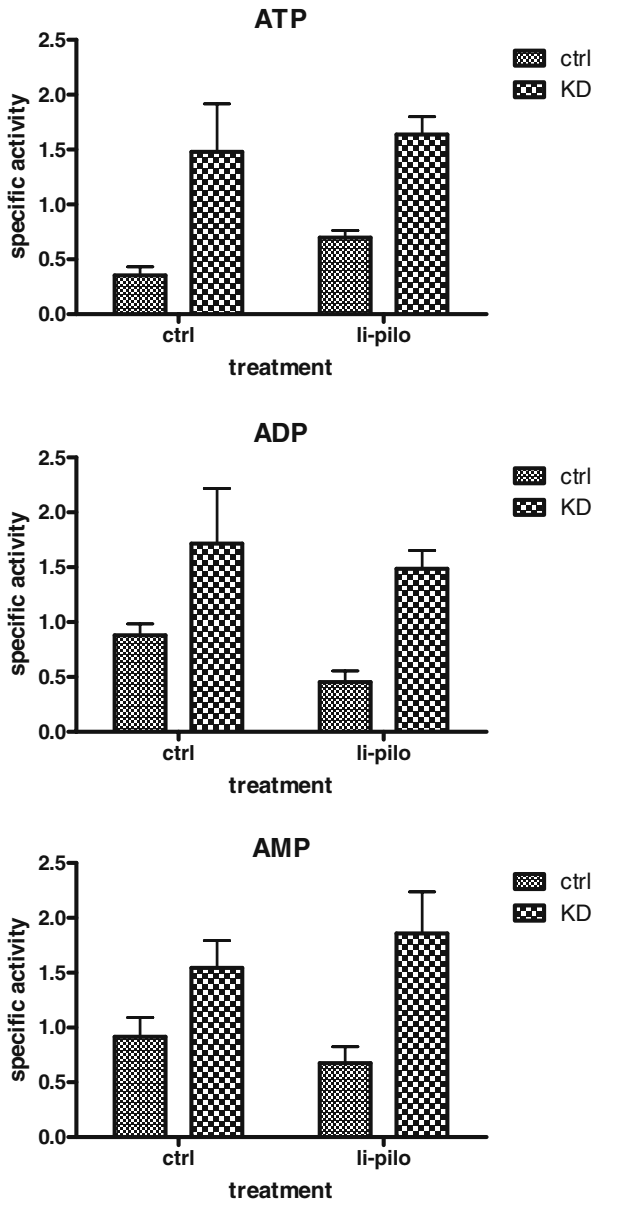

Fig. 1 ATP, ADP and AMP hydrolysis in rat blood serum. The animals were divided into four groups: Ctrl (received control diet); KD (received ketogenic diet); Li-Pilo (submitted to lithium–pilocarpine model and received control diet); and Li-Pilo+KD (submitted to lithium–pilocarpine model and received ketogenic diet). *Bars* represent means±S.D. of at least five animals. The results were analyzed by two-way ANOVA followed by Bonferroni post hoc test (GraphPad Prism vs 5)

increased ATP, ADP and AMP ($P<0.001$) hydrolysis, while lithium–pilocarpine-induced SE increased ATP hydrolysis ($P<0.001$) and decreased ADP hydrolysis ($P<0.001$). The lithium–pilocarine plus KD group did not alter ATP, ADP, and AMP hydrolysis when compared to KD group.

To evaluate the possible liver damage, we measured the levels of the hepatic enzymes ALP, ALT and AST in rat blood serum (Fig. 2). The statistical analysis showed that there was an interaction of KD on enzymes ALP, ALT and AST activities, as well interaction of SE on ALP and AST activities. There is no significant difference in ALP, ALT, and AST in lithium–pilocarpine plus KD group when compared with KD group.

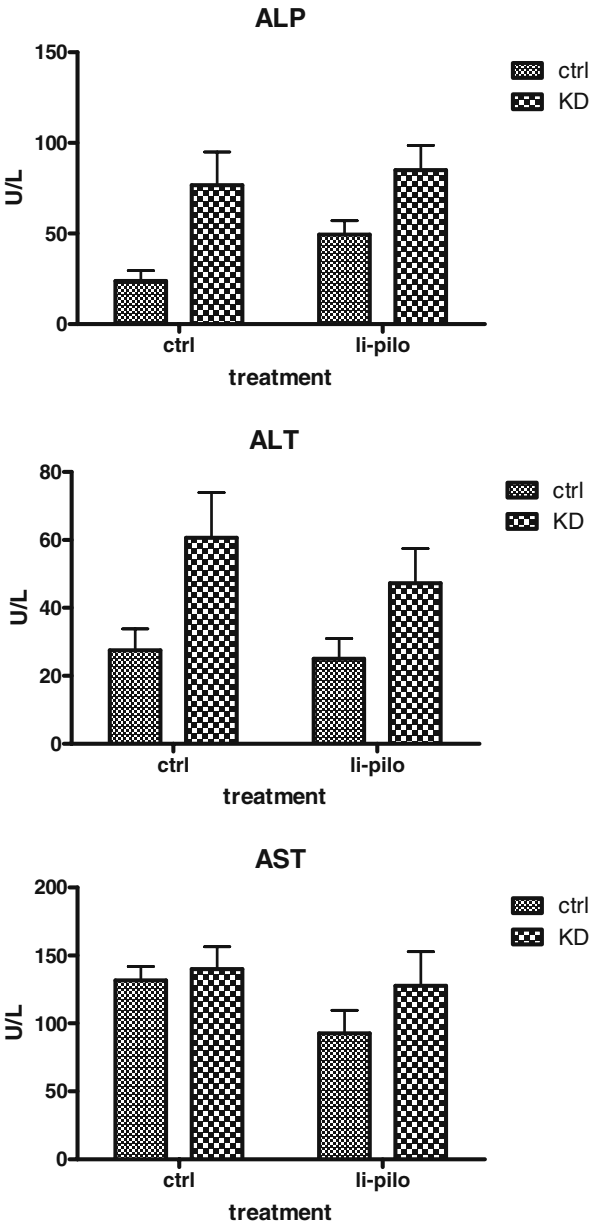

Fig. 2 Alkaline phosphatase (ALP), Alanine aminotranferase (ALT) and Aspartate aminotranferase (AST) activities in blood serum of rats. The animals were divided into four groups: Ctrl (received control diet); KD (received ketogenic diet); Li-Pilo (submitted to lithium–pilocarpine model and received control diet); and Li-Pilo+KD (submitted to lithium–pilocarpine model and received ketogenic diet). *Bars* represent means±S.D. of at least five animals. The results were analyzed by two-way ANOVA followed by Bonferroni post hoc test (GraphPad Prism vs 5)

Discussion

Previous studies from our group have analyzed the interaction between epilepsy and nucleotide hydrolysis in synaptosomal preparations at different periods after induction of SE by pilocarpine or kainate (Bonan et al. 2000; Oses et al. 2007). Walton et al. (1998) showed that after 2 h of continuous high-amplitude rapid spiking on EEG, there was a significant decrease in residual ATP levels in the homogenate and mitochondria fractions from *status epilepticus* rat brains compared to matched controls. However, no difference in residual ATP level was observed in the synaptosomal preparations of *status epilepticus* animals compared to controls (Walton et al. 1998). In addition, Bruno et al. (2002) reported an increase in the nucleotidase activities in rat blood serum after a single convulsive injection of pentylenetetrazol. In this second study, it was suggested that the increase in nucleotide hydrolysis was promoted by the seizures, since no significant changes in ATP and ADP hydrolysis were observed when the nucleotidases were analyzed in vitro and in the presence of PTZ, the agent inductor of seizure (Bruno et al. 2002).

In the present study, we used the lithium–pilocarpine model of epilepsy for induction of SE to investigate the effect of the ketogenic diet (KD) on nucleotide hydrolysis in the blood serum of rats. Considering that serum nucleotidases have been associated with the plastic changes induced by SE it seems to be important to evaluate the correlation between these two parameters. The present study demonstrates that lithium–pilocarpine-induced SE is able to increase ATP hydrolysis and decrease ADP hydrolysis in the blood serum of rats. In agreement with results from our laboratory, pilocarpine caused an increase in ATP, ADP and AMP hydrolysis in the blood serum of rats (Bruno et al. 2002). In the present study, we suggest that a single dose of lithium was able to inhibit the effect of pilocarpine on ADP hydrolysis, but was not sufficient to inhibit the increase in ATP hydrolysis caused by pilocarpine. Further studies are necessary, however, to investigate the mechanism of inhibition of nucleotidases by lithium in the blood serum.

It has been reported that KD may cause some metabolic changes, induced by high circulating levels of ketone bodies or perhaps by the lipid component of this diet. In fact, clinical studies have shown that children treated with KD could develop liver damage (Ballaban-Gil et al. 1998) and hepatits (Kang et al. 2004). Considering these data, we have evaluated the ALP, AST and ALT enzymes as classic markers for hepatic damage. The serum ALP is a sensitive detector for early intrahepatic and extrahepatic bile obstruction and the presence of infiltrative diseases of the liver (Gutman 1959), although it is sensitive for the diagnosis of liver disease, ALP activity is not specific (Reichling and Kaplan 1988). In contrast, 5′-NT is found in the liver as well as in other tissues (Goldberg 1973), but an increase in its serum activity has been shown to be specific for the liver and biliary tree (Hill and Sammons 1967; Song et al. 1969).

Similarly, aminotransferases are sensitive indicators of liver cell damage for both acute and chronic hepatocellular injury (Barth et al. 1979; Ludwig and Kaplowitz 1980). Giannini et al. (2003) suggested that the AST/ALT ratio determination could be useful in well-defined clinical situations due to its relationships with the stage of hepatic disease (both histological and clinical) and its correlation with liver function. Since our results showed an increase in ALP, aminotransferases and nucleotidases, especially 5′-NT, activities, we may propose that prolonged use of KD can cause hepatic disorders.

Another interesting finding of the present study is the increased ALP activity seen in epileptic rats, indicating a neurological damage induced by the lithium–pilocarpine model, as was recently shown by Thompson et al. (2006) who reported a rare syndrome known as hyperphosphatasia with neurologic deficit.

In summary, data reported herein show that seizures induced by lithium–pilocarpine elicit a significant increase in ATP hydrolysis and a decrease in ADP hydrolysis in the blood serum of rats. Although the protective actions of KD against epilepsy are not entire clearly identified, extracellular ATP and adenosine has been recently proposed as key molecules underlying the benefits and metabolic changes associated with the anticovulsant/neuroprotective effects of this diet (Masino and Geiger 2008, 2009). Furthermore, although the KD is a useful and effective agent in management of refractory epilepsies of childhood, it is a rigorous regimen that could be associated with hepatic damage, as indicated by our results through the elevated activities of ALP, aminotransferases and nucleotidases, especially 5′-NT, in the blood serum.

Acknowledgments This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Vanessa Gass da Silveira was recipient of a CNPq fellowship. We thank Jean Pierre Oses for the critical reading of the manuscript.

References

Ballaban-Gil K, Callahan C, O'Dell C, Pappo M, Moshé S, Shinnar S (1998) Complications of the ketogenic diet. Epilepsia 39(7): 744–748

Barth RC, Steven AS, Robert WS (1979) Clinical internal medicine. Little Brown, England, pp 211–214

Bonan CD, Walz R, Pereira GS, Worm PV, Battastini AM, Cavalheiro EA, Izquierdo I, Sarkis JJ (2000) Changes in synaptosomal ectonucleotidase activities in two rat models of temporal lobe epilepsy. Epilepsy Res 39(3):229–238

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 72:218–254

Bruno AN, Oses JP, Bonan CD, Walz R, Battastini AM, Sarkis JJ (2002) Increase of nucleotidase activities in rat blood serum after a single convulsive injection of pentylenetetrazol. Neurosci Res 43(3):283–288

Bruno AN, Oses JP, Amaral O, Coitinho A, Bonan CD, Battastini AM, Sarkis JJ (2003) Changes in nucleotide hydrolysis in rat blood serum induced by pentylenetetrazol-kindling. Brain Res: Mol Brain Res 114(2):140–145

Cavalheiro EA (1995) The pilocarpine model of epilepsy. Ital J Neurol Sci 16:33–37

Cavalheiro EA, Delrio FS, Turski WA, Calderazzo-Filho LS, Bortolotto ZA, Turski L (1987) The susceptibility of rats to pilocarpine-induced seizures is age-dependent. Brain Res 26:32–47

Chan K, Delfert D, Junger KD (1986) A direct colorimetric assay for Ca2+-ATPase activity. Anal Biochem 157:375–380

Chen W, Guidotti G (2001) The metal coordination of sCD39 during ATP hydrolysis. BMC Biochem 2:9

Dixon TF, Purdom M (1954) Serum 5′-nucleotidase. J Clin Pathol 7:341–343

Dubé C, da Silva Fernandes MJ, Nehlig A (2001a) Age-dependent consequences of seizures and the development of temporal lobe epilepsy in the rat. Dev Neurosci 23:219–223

Dubé C, Boyet S, Marescaux C, Nehlig A (2001b) Relationship between neuronal loss and interictal glucose metabolism during the chronic phase of the lithium–pilocarpine model of epilepsy in the immature and adult rat. Exp Neurol 167:227–241

Dubyak GR (2000) Purinergic signaling at immunological synapses. J Auton Nerv Syst 81(1–3):64–68

Giannini E, Risso D, Botta F, Chiarbonello B, Fasoli A, Malfatti F, Romagnoli P, Testa E, Ceppa P, Testa R (2003) Validity and clinical utility of the aspartate aminotransferase-alanine aminotransferase ratio in assessing disease severity and prognosis in patients with hepatitis C virus-related chronic liver disease. Arch Int Med 163(2):218–224

Goldberg DM (1973) 5′nucleotidase: recent advances in cell biology, methodology and clinical significance. Digestion 8:87–99

Gutman AB (1959) Serum alkaline phosphatase activity in diseases of the skeletal and hepatobiliary systems. A consideration of the current status. Am J Med 27:875–901

Hill PG, Sammons HG (1967) An assessment of 5′nucleotidase as a liver function test. Q J Med 36:457–468

Honchar MP, Olney JW, Sherman WR (1983) Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. Science 220:323–325

Janigro D (1999) Blood–brain barrier, ion homeostatis and epilepsy: possible implications towards the understanding of ketogenic diet mechanisms. Epilepsy Res 37:223–232

Leite JP, Garcia-Cairasco N, Cavalheiro EA (2002) New insights from the use of pilocarpine and kainate models. Epilepsy Res 50:93–103

Ludwig S, Kaplowitz N (1980) Effect of pyridoxine deficiency on serum and liver transaminases in experimental liver injury in the rat. Gastroenterology 79(3):545–549

Kang HC, Chung DE, Kim DW, Kim HD (2004) Early- and late-onset complications of the ketogenic diet for intractable epilepsy. Epilepsia 45(9):1116–1123

Masino SA, Geiger JD (2008) Are purines mediators of the anticonvulsivant/neuroprotective effects of ketogenic diet? Trends Neurosci 31(6):273–278

Masino SA, Geiger JD (2009) The ketogenic diet and epilepsy: is adenosine the missing link? Epilepsia 50(2):332–333

Morrisett RA, Jope RS, Snead OC (1987a) Effects of drugs on the initiation and maintenance of status epilepticus induced by administration of pilocarpine to lithium-pretreated rats. Exp Neurol 97:193–200

Morrisett RA, Jope RS, Snead OC (1987b) Status epilepticus is produced by administration of cholinergic agonists to lithium-treated rats: comparison with kainic acid. Exp Neurol 98(3):594–605

Neuschwander-Tetri BA, Nicholson C, Tracy WLD, Jr TF (1996) Cholestatic liver injury down-regulates hepatic glutathione synthesis. J Surg Res 63(2):447–451

Oses JP, Cardoso CM, Germano RA, Kirst IB, Rücker B, Fürstenau CR, Wink MR, Bonan CD, Battastini AM, Sarkis JJF (2004) Soluble NTPDase: an additional system of nucleotide hydrolysis in rat blood serum. Life Sci 74(26):3275–3284

Oses JP, Viola GG, de Paula CG, Júnior VH, Hansel G, Böhmer AE, Leke R, Bruno AN, Bonan CD, Bogo MR, Portela LV, Souza DO, Sarkis JJ (2007) Pentylenetetrazol kindling alters adenine and guanine nucleotide catabolism in rat hippocampal slices and cerebrospinal fluid. Epilepsy Res 75(2–3):104–111

Pagani F, Panteghini M (2001) 5′-nucleotidase in the detection of increased activity of the liver form of alkaline phosphatase in serum. Clin Chem 47(11):2046–2048

Reichling J, Kaplan M (1988) Clinical use of serum enzymes in liver disease. Dig Dis Sci 33(12):1601–1614

Reitman S, Frankel SA (1957) Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56–63

Rigoulot MA, Koning E, Ferrandon A, Nehlig A (2004) Neuroprotective properties of topiramate in the lithium–pilocarpine model of epilepsy. J Pharmacol Exp Ther 308(2):787–795

Roberts WM (1930) Variations in the phosphatase activity in the blood in disease. Br J Exp Pathol 11:90–95

Roy AV (1970) Rapid method for determining alkaline phosphatase activity in serum with thymolphthalein monophosphate. Clin Chem 16(5):431–436

Schwartzkroin PA (1999) Mechanisms underlying the anti-epileptic efficacy of the ketogenic diet. Epilepsy Res 37:171–180

Song CS, Kappas A, Bodansky O (1969) 5′nucleotidase of plasma membranes of the rat liver: studies and subcellular distribution. Ann N Y Acad Sci 166:565–573

Stafstrom CE (2004) Dietary approaches to epilepsy treatment: old and new options on the menu. Epilepsy Curr 4(6):215–222

Thompson MD, Killoran A, Percy ME, Nezarati M, David EC, Cole DEC, Hwang PA (2006) Hyperphosphatasia with neurologic deficit: a pyridoxine-responsive seizure disorder. Pediatr Neurol 34(4):303–307

Todorov LD, Mihaylova-Todorova S, Westfall TD, Sneddon P, Kennedy C, Bjur RA, Westfall DP (1997) Neuronal release of soluble nucleotidases and their role in neurotransmitter inactivation. Nature 387:76–79

Turski WA (2000) Pilocarpine-induced seizures in rodents-17 years on. Polish J Pharmacol 52:63–65

Turski L, Ikonomidou C, Turski WA, Bortolotto ZA, Cavalheiro EA (1989) Review: cholinergic mechanisms and epileptogenesis. The seizures induced by pilocarpine: a novel experimental model of intractable epilepsy. Synapse 3:154–171

Walton NY, Nagy AK, Treiman DM (1998) Altered residual ATP content in rat braincortex subcellular fractions following status epilepticus induced by lithium and pilocarpine. J Mol Neurosci 11(3):233–242

Wilder RM (1921) The effects of ketonemia on the course of epilepsy. Mayo Clin Proc 2:307–308

Yegutkin GG (1997) Kinetic analysis of enzymatic hydrolysis of ATP in human and rat blood serum. Biochemistry-Moscow 62:724–728

Yegutkin GG, Bodin P, Burnstock G (2000) Effect of shear stress on the release of soluble ecto-enzymes ATPase and 5′-nucleotidase along with endogenous ATP from vascular endothelial cells. Brit J Pharmacol 129(5):921–926

Zhao Q, Stafstrom CE, Fu DD, Hu Y, Holmes GL (2004) Detrimental effects of the ketogenic diet on cognitive function in rats. Pediatr Res 5(3):498–506

Zhou SL, Gordon RE, Bradbury M, Stump D, Kiang CL, Berk PD (1998) Ethanol up-regulates fatty acid uptake and plasma membrane expression and export of mitochondrial aspartate aminotransferase in HepG2 cells. Hepatology 27(4):1064–1074

Zimmermann H (2001) Ectonucleotidases: some recent developments and a note on nomenclature. Drug Dev Res 52:44–56