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Postictal serum nucleotidases activities in patients with epilepsy

Salvatore Grosso[a,*], **Raffaele Rocchi**[b], **Maria Margollicci**[a], **Giampaolo Vatti**[a], **Alice Luddi**[a], **Francesca Marchi**[b], **Paolo Balestri**[a]

[a] *Department of Pediatrics, Pediatric Neurology Section, University of Siena, Siena, Italy*
[b] *Department of Neuroscience, University of Siena, Siena, Italy*

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Summary Adenosine, a potent anticonvulsant, can be produced in the body by the hydrolysis of adenine nucleotides through the action of ecto- or soluble nucleotidases. Changes in nucleotide hydrolysis occur after pentylenetetrazol-induced epileptic events. We evaluated serum ATP, ADP and AMP hydrolysis rates and soluble nucleotide phosphodiesterase (PDEase) activity at 5, 10, 15, 30 and 60 min, and 12 h following an epileptic event. Fifteen patients (seven female, eight male; mean age 15.5 years) were included in the study. The type of seizure was generalized in four patients and was localization related in the remaining 11. There were no differences in adenine nucleotide hydrolysis rates between patients and healthy subjects in the interictal stage. In comparison with controls, ATP, ADP and AMP hydrolysis rates were significantly increased at 5 min ($53 \pm 1.4\%$, $79.2 \pm 2.8\%$ and $37.0 \pm 2.6\%$, respectively) and up to 30 min following the epileptic event. In contrast to ADP and AMP, ATP hydrolysis remained significantly increased at 60 min ($71.4 \pm 1.6\%$), returning to the basal level after 12 h. Serum PDEase activity was also significantly higher in the patients than in healthy subjects, peaking at 15 min ($61 \pm 2.9\%$) and remaining significantly increased up to 60 min ($4.6 \pm 1.2\%$) following the epileptic episode. Globally, the variations in the postictal serum ADP hydrolysis rate almost overlapped those of AMP hydrolysis, whereas changes in the ATP hydrolysis rate overlapped those of PDEase activity.

The clinical significance of this elevation in postictal soluble serum nucleotidase activity remains to be clarified. However, it is possible to hypothesize that the higher nucleotidase activity might play a role in the modulation of epileptic events.

* Corresponding author at: Pediatric Neurology Section, Department of Pediatrics, Obstetrics, Gynecology, and Reproductive Medicine, University of Siena, ''S. Maria alle Scotte'' Hospital, 53100 Siena, Italy. Tel.: +39 0577 586546; fax: +39 0577 586143.
E-mail address: grosso@unisi.it (S. Grosso).

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Introduction

Adenine nucleotides such as ATP, ADP and AMP and their nucleoside derivative adenosine are important signaling molecules in several physiological and pathological processes (Ralevic and Burnstock, 2003; Boison, 2008a,b). Extracellular nucleotides exist normally at low concentrations, but in pathological situations, they can be released in large quantities. Platelets, vascular endothelium and red blood cells are able to release large amounts of adenine nucleotides into the blood. In the nervous system, ATP can be released by both neurons and astrocytes (Boison, 2008a,b; Todorov et al., 1997; Yegutkin, 2008). When released in the synaptic cleft, ATP is rapidly hydrolyzed to adenosine. Adenosine is a potent anticonvulsant (Boison, 2008b; Dragunow, 1986; Dunwiddie, 1999) and has neuroprotective properties (Cunha, 2005; Dragunow and Faull, 1988; Ribeiro, 2005). These effects are mainly exerted by activation of the high-affinity inhibitory adenosine A1 and excitatory adenosine A2 receptors (Dunwiddie and Masino, 2001; Fredholm et al., 2005a,b).

The role of ATP neurotransmission and gliotransmission in the pathophysiology of epileptic seizures has been recently reviewed (Kumaria et al., 2008). ATP and gap junctions are involved in the propagation of the glial calcium wave which have recently been demonstrated to underlie seizures. In this perspective, blocking ATP mediated gliotransmission may represent a potential target for antiepileptic drugs. Kumaria et al. (2008) hypothesized that blockade of gliotransmission by purinergic modulation may have positive effects on epilepsy by decreasing synaptic strength across the tripartite synapse and by preventing synchronous ictal spread to distant sites. Moreover, it is possible that ATP released by astrocytes in response to neuronal activity is involved in the surround inhibition to adjacent neurons. Such a model would both prevent propagation of seizures and also enhance neurotransmission by decreasing background noise (Kumaria et al., 2008).

Enzymes involved in adenine nucleotide metabolism can be either anchored to cell membranes (ecto-enzymes) or soluble in body fluids. They include: (i) the nucleoside triphosphate diphosphohydrolase (NTPDase) family; (ii) the nucleoside pyrophosphatase/phosphodiesterase (PDEase) family and (iii) the 5′-nucleotidase (Zimmermann, 2001). These nucleotidases control the availability of ligands (ATP, ADP, AMP and adenosine) for both nucleotide and nucleoside receptors and, consequently, the duration and extent of receptor activation (Chen and Guidotti, 2001).

Considering that soluble nucleotidases are able to produce adenosine as well, we investigated the postictal variation in ATP, ADP, AMP hydrolysis rates and PDEase activity in the serum of patients suffering from epilepsy.

Patients and methods

Subjects

Two groups of subjects were included in the study. Group 1 consisted of 15 patients affected by epilepsy (seven female and eight male) with a mean age of 15.5 years (range 8.5—32.5). The patients were selected from those who were undergoing 24 h inpatient video and electroencephalography (EEG) monitoring for the electroclinical characterization of seizures, localization of the seizure focus, or therapeutic assessment. Seizures and epileptic syndromes were classified according to the ILAE diagnostic criteria (Commission, 1981, 1989). Patients with neurological progressive diseases and cardiovascular disorders were excluded. Family and personal histories were registered, and neurological examinations were performed on all patients. All patients underwent imaging studies with brain magnetic resonance imaging (MRI). Biochemical analyses, chromosomal investigations and screening for metabolic disorders were carried out on all patients. Four patients were classified as having generalized epilepsy, and the remaining 11 presented with localization-related epilepsy. Three of the patients were on antiepileptic drug monotherapy, and 12 were on polytherapy (median number of medications = 2). Group 2 consisted of 15 healthy subjects matched for age and gender with Group 1, who came in for an annual checkup at the Department of Pediatrics and Department of Neuroscience of the University of Siena, Italy. None of these subjects had suffered from epilepsy, neurological or cardiovascular disorders. Informed consent for blood withdrawal was obtained from all subjects or from their parents as appropriate.

Blood sampling and biochemical analysis

Healthy subjects and patients in their interictal stage were evaluated after an overnight fast. One hour before the study, the healthy subjects entered a quiet room, and the patients were admitted for video-EEG monitoring. Both groups of subjects were placed on bed rest. An indwelling gauge catheter was placed in an antecubital vein, and care was taken to obtain and treat all blood samples. After 1 h of accommodation, blood was taken through the indwelling catheter for analysis (baseline levels or time '0'). Epileptic patients were seizure free for at least 12 h before blood withdrawal, and blood samples were obtained postictally after 5, 10, 15, 30 and 60 min, and at 12 h after the index seizure, which was defined as the first clearly identifiable ictal event. All serum samples were stored at −70 °C before analysis.

Measurement of ATP, ADP and AMP hydrolysis

Hydrolysis rates of ATP, ADP and AMP were evaluated as reported (Bruno et al., 2002; Yegutkin, 1997). Briefly, the reaction mixture, which contained 5 mM $CaCl_2$ and 44 mM Tris—HCl pH 8.0 (pH 7.2 for the measurement of AMP hydrolysis) in a final volume of 175 μL, was preincubated with 5 μL of serum for 10 min at 37 °C. The reaction was initiated by the addition of 10 μL ATP, or ADP or AMP, each to a final concentration of 1.0 mM, incubated for 10 min at 37 °C and stopped with 200 μL of 10% trichloroacetic acid (TCA). Incubation times were chosen to ensure the linearity of the reaction. Inorganic phosphate (Pi) released was determined as described (Chan and Swaminathan, 1986). Light absorbance was measured at 630 nm. Controls to correct for nonenzymatic hydrolysis were performed by adding the serum after the reaction was stopped with TCA. All samples were evaluated in triplicate. Enzyme activities were expressed as nanomoles of Pi released per minute per milliliter of serum.

Measurement of p-Nph-5′-TPM hydrolysis

PDEase activity was determined as described by Sakura et al. (1998). The reaction mixture containing 0.5 mM p-nitrophenil-5′-thymidine-monophosphate (p-Nph-5′-TMP) as a substrate in 40 mM Tris—HCl (pH 8.9) was preincubated at 37 °C for 10 min in a final volume of 230 μL. The reaction was initiated by the addition of 20 μL serum, incubated for 10 min at 37 °C and stopped with 750 μL of 0.2 M NaOH. Incubation times were chosen to ensure the linearity of the reaction. The amount of p-nitrophenol was measured by light absorbance at 400 nm. Controls to correct for nonenzymatic hydrolysis were performed by adding the serum after the reaction was stopped with