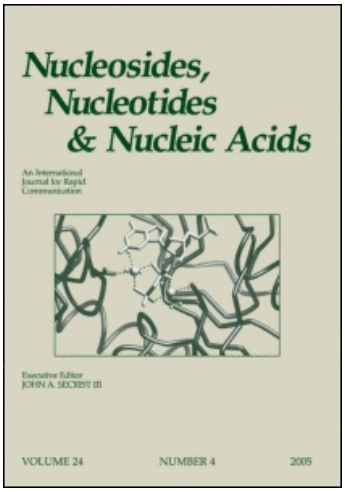

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 27 October 2004

To cite this Article Pesi, R. , Camici, M. , Micheli, V. , Notarantonio, L. , Jacomelli, G. and Tozzi, M. G.(2004) 'Identification of the 5'-Nucleotidase Activity Altered in Neurological Syndromes', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1257 — 1259
To link to this Article: DOI: 10.1081/NCN-200027530
URL: http://dx.doi.org/10.1081/NCN-200027530

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1257–1259, 2004

Identification of the 5′-Nucleotidase Activity Altered in Neurological Syndromes

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ABSTRACT

5′-nucleotidases comprise a family of enzymes involved in the regulation of intracellular and extracellular nucleotide concentration. There is increasing knowledge about an involvement of these activities in the aetiology of neurological disorders. In this paper we present a protocol for the identification of the altered enzyme in fibroblasts primary culture from patients and controls.

Key Words: 5′-nucleotidase; AMP hydrolysis; Fibroblast; Neurological disorders.

INTRODUCTION

Nucleoside monophosphate phosphohydrolases comprise a family of enzymes that dephosphorylate nucleotides both in intracellular and extracellular compartments.[1] Members of this family exhibit different sequence, location, substrate specificity and regulation. The genes encoding for these proteins are located in distinct chromosomes. Besides the ectosolic 5′-nucleotidase, several cytosolic (cN-I, cN-II, pN-I, dNT-I) and one mitochondrial enzymes (dNT-II) have been described, presumably these proteins have

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DOI: 10.1081/NCN-200027530
1525-7770 (Print); 1532-2335 (Online)

different physiological roles.[2–5] Nevertheless, several researchers refer to as 5′-nucleotidase any AMP-dephosphorylating activity, lacking a more accurate identification. An increase of cytosolic 5′-nucleotidase activity has been associated with neurological disorders.[6–8] The identification of the specific enzyme involved in these pathologies would be fundamental for the comprehension of the linkage between the alteration of enzyme activity and alteration of brain functions.

MATERIALS AND METHODS

Patients have been chosen on the base of their symptoms including autistic features, speech retardation and their impaired urate/creatinine ratio in urine. Primary cultures of fibroblasts from healthy subjects were used as controls. Human skin fibroblasts were obtained after informed consent, and were grown at 37°C in a humidified CO2 atmosphere in RPMI medium supplemented with L-glutamine, antibiotics and 10% foetal calf serum until confluence. We measured the activity of cN-I and ectosolic nucleotidase as the rate of radiolabelled AMP hydrolysis in the presence or in the absence of 470 μM α,β-methyleneadenosine 5′-diphosphate (AMP-PC) and 20 mM magnesium, cN-II as the rate of phosphotransfer from IMP to radiolabelled inosine[9] and dNTI and II as the rate of radiolabelled dUMP hydrolysis.[10]

RESULTS

We present here a relatively simple protocol which enabled us to measure the specific activity of three different nucleotidases in primary cultures of fibroblasts of patients and controls.

Our preliminary results indicate that the described neurological symptoms, in at least two cases, are associated with an increase of AMP hydrolysing activity presenting functional characteristics compatible with ectosolic 5′-nucleotidase, since it is inhibited by micromolar concentration of AMP-PC and is not totally dependent on magnesium. Surprisingly, in fibroblasts of patients showing nucleotidase hyperactivity, adenylate pool was remarkably depleted, suggesting an increase of the intracellular adenylate catabolism.

DISCUSSION

In our hands the suggested protocol conducted to the identification of the enzyme responsible of the increase of AMP hydrolysis in two patients also showing a remarkable depletion of adenylate compounds. We suggest that a still unidentified mutation can cause the expression inside the cell of a solubilized form of ectosolic 5′-nucleotidase.

REFERENCES

1. Zimmermann, H.; Biochem, J. 5′-Nucleotidase: molecular structure and functional aspects. Biochem. J. **1992**, *285*, 345–365.

2. Sala-Newby, G.B.; Skladanowsky, A.C.; Newby, A.C. The mechanism of adenosine formation in cells. Cloning of cytosolic 5′-nucleotidase-I. J. Biol. Chem. **1999**, *274*, 17789–17793.
3. Allegrini, S.; Scaloni, A.; Ferrara, L.; Pesi, R.; Pinna, P.; Sgarrella, F.; Camici, M.; Eriksson, S.; Tozzi, M.G. Bovine cytosolic 5′-nucleotidase acts through the formation of an aspartate 52-phosphoenzyme intermediate. J. Biol. Chem. **2001**, *276*, 33526–33532.
4. Rampazzo, C.; Johansson, M.; Gallinaro, L.; Ferraro, P.; Hellman, U.; Karlsson, A.; Reichard, P.; Bianchi, V. Mammalian 5′(3′)-deoxyribonucleotidase, cDNA cloning, and overexpression of the enzyme in Escherichia coli and mammalian cells. J. Biol. Chem. **2000**, *275*, 5409–5415.
5. Rampazzo, C.; Gallinaro, L.; Milanesi, E.; Frigimelica, E.; Reichard, P.; Bianchi, V. A deoxyribonucleotidase in mitochondria: involvement in regulation of dNTP pools and possible link to genetic disease. PNAS **2000**, *97*, 8239–8244.
6. Page, T.; Yu, A.; Fontanesi, J.; Nyhan, W.L. Developmental disorder associated with increased cellular nucleotidase activity. PNAS **1997**, *94*, 11601–11606.
7. Pesi, R.; Micheli, V.; Jacomelli, G.; Peruzzi, L.; Camici, M.; Garcia-Gil, M.; Allegrini, S.; Tozzi, M.G. Cytosolic 5′-nucleotidase hyperactivity in erythrocytes of Lesch-Nyhan syndrome patients. NeuroReport **2000**, *11*, 1827–1831.
8. Garcia-Gil, M.; Pesi, R.; Perna, S.; Allegrini, S.; Giannecchini, M.; Camici, M.; Tozzi, M.G. 5′-aminoimidazole-4-carboxamide riboside induces apoptosis in human neuroblastoma cells. Neuroscience **2003**, *117*, 811–820.
9. Tozzi, M.G.; Camici, M.; Pesi, R.; Allegrini, S.; Sgarrella, F.; Ipata, P.L. Nucleoside phosphotransferase activity of human colon carcinoma cytosolic 5′-nucleotidase. Arch. Biochem. Biophys. **1991**, *291*, 212–217.
10. Rampazzo, C.; Mazzon, C.; Reichard, P.; Bianchi, V. 5′-nucleotidases: specific assays for five different enzymes in cell extracts. Biochim. Biophys. Res. Commun. **2002**, *293*, 258–263.