

Endosymbiotic Actinidic Archaeal Digoxin Inhibited Sodium Potassium ATPase Mediated ATP Synthesis and Archaeal Ectoatpases Produce Neuro-Immuno-Metabolic-Endocrine/Cell Cycle Regulation

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Abstract

Aims and Objectives: Actinidic archaea has been related to the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration. Archaea have got ecto ATPases. EctoATPases convert ATP to ADP and AMP. ATP functions as purinergic neurotransmitter regulating multiple cell functions. 5'AMP activates the metabolic gauge AMP kinase. Archaeal digoxin produces membrane sodium potassium ATPase inhibition. Membrane sodium potassium ATPase in the setting of digoxin induced inhibition can synthesize ATP. This ATP can serve as a substrate for ectoATPase and functions as a archaeal signalling molecule. The RBC membrane sodium potassium ATPase mediated ATP synthesis and archaeal ectoATPase activity was assessed in the above mentioned disorders.

Methodology: The following groups were included in the study:- endomyocardial fibrosis, alzheimer's disease, multiple sclerosis, non-hodgkin's lymphoma, metabolic syndrome x with cerebrovascular thrombosis and coronary artery disease, schizophrenia, autism, seizure disorder, creutzfeldt jakob disease and acquired immunodeficiency syndrome. Plasma from fasting heparinised blood was used and the experimental protocol was as follows (I) Plasma+phosphate buffered saline, (II) same as I+cholesterol substrate, (III) same as II+rutile 0.1 mg/ml, (IV) same as II+ciprofloxacin and doxycycline each in a concentration of 1 mg/ml. The following three estimations were carried out:- Cytochrome F420 and ectoATPase activity. RBCs from the patient's blood were separated within 1 hour of collection of blood and ATP synthesis

activity was assessed in the presence of added digoxin to produce a concentration 12.2 ng/dl.

Results: Plasma of control subjects showed increased levels of the above mentioned parameters with after incubation for 1 hour and addition of cholesterol substrate resulted in still further significant increase in these parameters. The plasma of patients showed similar results but the extent of increase was more. The addition of antibiotics to the control plasma caused a decrease in all the parameters while addition of rutile increased their levels. The addition of antibiotics and rutile to the patient's plasma produced the same changes but the extent of change was more in patient's sera as compared to controls. There was RBC membrane ATP synthesis in the patient population in the presence of added digoxin indicating membrane sodium potassium ATPase mediated ATP synthesis.

Conclusion: An actinide dependent shadow biosphere of archaea in the above mentioned disease states is described. There was RBC membrane sodium potassium ATPase mediated ATP synthesis and serum archaeal ectoATPase activity in the patients with schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration. The ectoATPase can degrade the RBC membrane synthesized ATP to 5'AMP which can activate AMPK producing metabolic integration. The membrane sodium potassium ATPase synthesized ATP acts as a purinergic transmitter regulating immunity, neural function and cell differentiation/death.

Key words: Actinide; Archaea; Extracellular ATP; Sodium potassium ATPase; EctoATPases; AMPK; purinergic transmission

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INTRODUCTION

Actinidic archaea has been related to the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration. Archaea have got ecto ATPases. EctoATPases convert ATP to ADP and AMP. ATP functions as purinergic neurotransmitter regulating multiple cell functions. 5'AMP activates the metabolic gauge AMP kinase. Archaeal digoxin produces membrane sodium potassium ATPase inhibition. Membrane sodium potassium ATPase in the setting of digoxin induced inhibition can synthesize ATP. This ATP can serve as a substrate for ectoATPase. This ATP can serve as a substrate for ectoATPase and functions as a archaeal signalling molecule. The RBC membrane sodium potassium ATPase mediated ATP synthesis and archaeal ectoATPase activity was assessed in the above mentioned disorders^[1-9].

MATERIALS AND METHODS

The following groups were included in the study:- endomyocardial fibrosis, alzheimer's disease, multiple sclerosis, non-hodgkin's lymphoma, metabolic syndrome x with cerebrovascular thrombosis and coronary artery disease, schizophrenia, autism, seizure disorder, creutzfeldt jakob disease and acquired immunodeficiency syndrome. There were 10 patients in each group and each patient had an age and sex matched healthy control selected randomly from the general population. The blood samples were drawn in the fasting state before treatment was initiated. Plasma from fasting heparinised blood was used and the experimental protocol was as follows (I) Plasma+phosphate buffered saline, (II) same as I+cholesterol substrate, (III) same as II+rutile 0.1 mg/ml, (IV) same as II+ciprofloxacin and doxycycline each in a concentration of 1 mg/ml. Cholesterol substrate was prepared as described by Richmond^[10]. Aliquots were withdrawn at zero time immediately after mixing and after incubation at 37°C for 1 hour. The following three estimations were carried out:- Cytochrome F420 and ectoATPase activity. RBCs from the patient's blood were separated within 1 hour of collection of blood and ATP synthesis activity was assessed in the presence of added digoxin to produce a concentration 12.2 ng/dl^[11-13]. Cytochrome F420 was estimated fluorimetrically (excitation wavelength 420 nm and emission wavelength 520 nm). Informed consent of the subjects and the approval of the ethics committee were obtained for the

study. The statistical analysis was done by ANOVA.

RESULTS

Plasma of control subjects showed increased levels of the above mentioned parameters with after incubation for 1 hour and addition of cholesterol substrate resulted in still further significant increase in these parameters. The plasma of patients showed similar results but the extent of increase was more. The addition of antibiotics to the control plasma caused a decrease in all the parameters while addition of rutile increased their levels. The addition of antibiotics to the patient's plasma caused a decrease in all the parameters while addition of rutile increased their levels but the extent of change was more in patient's sera as compared to controls. The results are expressed in tables 1-3 as percentage change in the parameters after 1 hour incubation as compared to the values at zero time. There was RBC membrane sodium potassium ATPase mediated ATP synthesis and serum archaeal ectoATPase activity in the patients with schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration.

Table 1
Effect of Rutile and Antibiotics on Cytochrome F420

Group	CYT F420 % (Increase with Rutile)		CYT F420 % (Decrease with Doxy+Cipro)	
	Mean	± SD	Mean	± SD
Normal	4.48	0.15	18.24	0.66
Schizo	23.24	2.01	58.72	7.08
Seizure	23.46	1.87	59.27	8.86
AD	23.12	2.00	56.90	6.94
MS	22.12	1.81	61.33	9.82
NHL	22.79	2.13	55.90	7.29
DM	22.59	1.86	57.05	8.45
AIDS	22.29	1.66	59.02	7.50
CJD	22.06	1.61	57.81	6.04
Autism	21.68	1.90	57.93	9.64
EMF	22.70	1.87	60.46	8.06
		F value 306.749	F value 130.054	
		P value < 0.001	P value < 0.001	

Table 2
Effect of Rutile and Antibiotics on Archaeal EctoATPase Activity

Group	EctoATPase activity % change (Increase with Rutile)		EctoATPase activity % change (Decrease with Doxy+Cipro)	
	Mean	± SD	Mean	± SD
Normal	4.34	0.15	18.24	0.37
Schizo	23.02	1.65	67.61	2.77
Seizure	22.13	2.14	66.26	3.93
AD	23.09	1.81	65.86	4.27
MS	21.93	2.29	63.70	5.63
NHL	23.12	1.71	65.12	5.58
DM	22.73	2.46	65.87	4.35
AIDS	22.98	1.50	65.13	4.87
CJD	23.81	1.49	64.89	6.01
Autism	22.79	2.20	64.26	6.02
EMF	22.82	1.56	64.61	4.95
F value 348.867		F value 364.999		
P value < 0.001		P value < 0.001		

Table 3
RBC Membrane ATP Synthesis in the Presence of Digoxin

Group	RBC membrane ATP synthesis %	
	Mean	± SD
Normal	4.40	0.11
Schizo	23.67	1.42
Seizure	23.09	1.90
AD	23.58	2.08
MS	23.52	1.76
NHL	24.01	1.17
DM	23.72	1.73
AIDS	23.15	1.62
CJD	23.00	1.64
Autism	22.60	1.64
EMF	23.37	1.31
F value 449.503		
P value < 0.001		

DISCUSSION

There was increase in cytochrome F420 indicating archaeal growth. The archaea can synthesise and use cholesterol as a carbon and energy source^[14,15,16]. The archeal origin of the enzyme activities was indicated by antibiotic

induced suppression. The study indicates the presence of actinide based archaea with an alternate actinide based enzymes or metalloenzymes in the system as indicated by rutile induced increase in enzyme activities^[14-16]. There was increased actinide dependent ectoATPase activity. The ectoATPase activity was stimulated by rutile and inhibited by antibiotics. The ectoATPase can convert extracellular ATP into ADP and 5'AMP. There was also evidence of RBC membrane ATP synthesis mediated by digoxin inhibited RBC membrane sodium potassium ATPase. The membrane sodium potassium ATPase in the setting of digoxin mediated inhibition can synthesize ATP. This extracellular ATP serves as a substrate for archaeal ectoATPases to generate AMP and ADP. The archaeal digoxin inhibits membrane sodium potassium ATPase and the inhibited enzyme synthesizes ATP which functions as a signaling molecule. The archaea regulates the neuro-immuno-metabolic-endocrine system using extracellular ATP.

ATP is a neurotransmitter in the central and sympathetic nervous system. ATP is stored in and released from synaptic nerve terminals and is known to act postsynaptically via P2X receptors. It promotes calcium signaling. ATP is a co-transmitter at noradrenaline, acetyl choline and GABA synapse. Extracellular ATP can inhibit glutamate transmission. It can thus regulate the NMDA/GABA thalamocorticothalamic pathway mediating conscious perception. ATP functions as a fast excitatory neurotransmitter and is involved in the pathogenesis of schizophrenia. It also plays a role in the pathogenesis of depression^[17-18].

Purinergic receptors regulate cell proliferation and cell differentiation. They also play a role in organ development and regeneration. Thus extracellular ATP may play a role in oncogenesis. Extra cellular ATP and purinergic receptors can mediate cell death. They can initiate neurodegenerative process. They are important in the pathogenesis of Alzheimer's disease and Parkinson's disease^[18,19].

Extracellular ATP and purinergic receptors are involved in immune activation and can initiate autoimmune disease. 5' AMP is immunosuppressive, inhibits NFkB and reduces cytokine secretion. ATP is immunostimulatory, activates NFkB and promotes cytokine secretion. Thus the ectoATPases can regulate the 5'AMP/ATP ratio and regulate immune signaling. Extracellular ATP can modulate the response to bacterial and viral infection. Extracellular ATP is involved in the signaling pathway of HIV infection and are important cell fusion and HIV replication. Extracellular ATP and purinergic receptors are also involved in lipopolysaccharide signaling^[20-22].

The ectoATPase acts upon ATP to generate 5'AMP. 5'AMP activates 5'AMP activated protein kinase. 5'AMP-activated protein kinase or AMPK or 5' adenosine monophosphate-activated protein kinase is an enzyme that

plays a role in cellular energy homeostasis. It is expressed in a number of tissues, including the liver, brain, and skeletal muscle. The net effect of AMPK activation is stimulation of hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis, stimulation of skeletal muscle fatty acid oxidation and muscle glucose uptake, and modulation of insulin secretion by pancreatic beta-cells. The *C. elegans* homologue of AMPK, *aak-2*, has been shown by Ristow and colleagues to be required for extension of life span in states of glucose restriction mediating a process named mitohormesis. 5'AMP can reversibly inhibit mitochondrial oxidative phosphorylation and produce cell hibernation. Purinergic receptors are involved in regulating pancreatic insulin and glucagon secretion. Extracellular ATP may play a role in the development of metabolic syndrome x^[23-25].

Thus the archaeal digoxin can mediate sodium potassium ATPase mediated extracellular ATP synthesis. The extracellular ATP is a archaeal signaling molecule. The extracellular ATP gets acted upon by actinide dependent archaeal ectoATPases to generate ADP and AMP. ATP can act upon purinergic receptors regulating neurotransmission, cell death, cell proliferation, cell differentiation, immunity, endocrine function and metabolism. 5'AMP can activate AMPK the principal metabolic gauge of the body.

REFERENCES

- [1] Hanold, D. & Randies, J. W. (1991). Coconut Cadang-Cadang Disease and Its Viroid Agent. *Plant Disease*, 75, 330-335.
- [2] Valiathan, M. S., Somers, K., Kartha, C.C. (1993). *Endomyocardial Fibrosis*. Delhi: Oxford University Press.
- [3] Edwin, B.T. & Mohankumaran, C. (2007). Kerala Wilt Disease Phytoplasma: Phylogenetic Analysis and Identification of a Vector. *Proutista Moesta, Physiological and Molecular Plant Pathology*, 71(1-3), 41-47.
- [4] Kurup, R. & Kurup, P. A. (2009). *Hypothalamic Digoxin, Cerebral Dominance and Brain Function in Health and Diseases*. New York: Nova Science Publishers.
- [5] Eckburg, P. B., Lepp, P. W., Relman, D. A. (2003). Archaea and Their Potential Role in Human Disease. *Infect Immun*, 71, 591-596.
- [6] Smit, A. & Mushegian, A. (2000). Biosynthesis of Isoprenoids via Mevalonate in Archaea: The Lost Pathway. *Genome Res*, 10(10), 1468-84.
- [7] Adam, Z. (2007). Actinides and Life's Origins, *Astrobiology*, 7, 6-10.
- [8] Schoner, W. (2002). Endogenous Cardiac Glycosides, a New Class of Steroid Hormones. *Eur J Biochem*, 269, 2440-2448.
- [9] Davies, P. C. W., Benner, S. A., Cleland, C. E., Lineweaver, C.H., McKay, C.P., Wolfe-Simon, F. (2009). Signatures of a Shadow Biosphere. *Astrobiology*, 10, 241-249.
- [10] Richmond, W. (1973). Preparation and Properties of a Cholesterol Oxidase from *Nocardia* Species and Its Application to the Enzymatic Assay of Total Cholesterol in Serum. *Clin Chem*, 19, 1350-1356.
- [11] Snell, E. D. & Snell, C. T. (1961). *Colorimetric Methods of Analysis* (Vol. 3A). New York: Van Nostrand.
- [12] Glick, D. (1971). *Methods of Biochemical Analysis* (Vol. 5). New York: Interscience Publishers.
- [13] Colowick, Kaplan, N. O. (1955). *Methods in Enzymology* (Vol. 2). New York: Academic Press.
- [14] Van der Geize R., Yam, K., Heuser, T., Wilbrink, M.H., Hara, H., Anderton, M. C. (2007). A Gene Cluster Encoding Cholesterol Catabolism in a Soil Actinomycete Provides Insight into Mycobacterium Tuberculosis Survival in Macrophages. *Proc Natl Acad Sci USA*, 104(6), 1947-52.
- [15] Francis, A.J. (1998). Biotransformation of Uranium and Other Actinides in Radioactive Wastes. *Journal of Alloys and Compounds*, 271(273), 78-84.
- [16] Probian, C., Wülfing, A., Harder, J. (2003). Anaerobic Mineralization of Quaternary Carbon Atoms: Isolation of Denitrifying Bacteria on Pivalic Acid (2,2-Dimethylpropionic Acid). *Applied and Environmental Microbiology*, 69(3), 1866-1870.
- [17] Skaper, S. D., Debetto P., Giusti P. (2010). The P2X7 Purinergic Receptor: From Physiology to Neurological Disorders. *FASEB J*, 24(2), 337-45.
- [18] Burnstock, G. (2008). Purinergic Signalling and Disorders of the Central Nervous System. *Nature Reviews Drug Discovery*, 7, 575-590.
- [19] White, N. & Burnstock, G. (2006). P2 Receptors and Cancer. *Trends in Pharmacological Sciences*, 27(4), 211-217.
- [20] Junger, W.G. (2011). Immune Cell Regulation by Autocrine Purinergic Signaling. *Nature Reviews Immunology*, 11, 201-212.
- [21] Séror C., Melki M. T., Subra F., Raza S. Q., Bras M., Saïdi H., Nardacci R., Voisin L., Paoletti A., et al. (2011). Extracellular ATP Acts on P2Y2 Purinergic Receptors to Facilitate HIV-1 Infection. *J Exp Med*, 208(9), 1823-34.
- [22] Guerra, A. N., Fiset P. L., Pfeiffer Z.A., Quinchia-Rios, B.H., Prabhu U., Aga M., Denlinger L.C., Guadarrama A.G., Abozeid S., Sommer J.A., Proctor R.A., Bertics P.J. (2003). Purinergic Receptor Regulation of LPS-induced Signaling and Pathophysiology. *J Endotoxin Res.*, 9(4), 256-63.
- [23] Winder, W. W., Hardie, D. G. (1999). AMP-Activated Protein Kinase, a Metabolic Master Switch: Possible Roles in Type 2 Diabetes. *Am J Physiol*, 277(1 Pt 1), E1-10.
- [24] Stapleton, D., Mitchelhill, K. I., Gao G., Widmer J., Mitchell B.J., Teh T., House C.M., Fernandez C.S., Cox T., Witters L. A., Kemp B. E. (1996). Mammalian AMP-activated Protein Kinase Subfamily. *J Biol Chem*, 271(2), 611-4.
- [25] Petit, P., Loubatières-Mariani M. M., Keppens S., Sheehan, M. J. (1996). Purinergic Receptors and Metabolic Function. *Drug Development Research*, 39(3-4), 413-425.