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Endosymbiotic Actinidic Archaeal Generation of Ammonia and Thiocyanate Regulates Cell/Neuroimmunoendocrine System and Provides a Substrate for Archaeal Energetics

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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. Actinidic archaea use cholesterol as a carbon and energy source. Archaeal cholesterol catabolism mediated by archaeal cholesterol oxidase can produce cholesterol ring oxidation to generate pyruvate. Pyruvate is converted to glutamate and ammonia can be generated from it. Archaeal urease can act upon urea generating ammonia and thiocyanate. Ammonia and thiocyanate serves the purpose of cellular and neuroimmune endocrine regulation. The archaea are ammonia oxidizing and can use ammonia for their energetics. The archaeal urease activity related ammonia and thiocyanate synthesis as well as cholesterol oxidase activity generating pyruvate and ammonia was studied in schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration.

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+ciprofloxacine and doxycycline each in a concentration of 1 mg/ml. The following estimations were carried out:- Cytochrome F420, hydrogen peroxide, pyruvate, ammonia, glutamate, thiocyanate and urease activity.

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.

Conclusion: An actinide dependent shadow biosphere of archaea and viroids in the above mentioned disease states is described. The archaeal urease generates thiocyanate and ammonia. The archaeal cholesterol oxidase catabolises cholesterol to generate pyruvate which is converted to glutamate and ammonia. Ammonia functions as a possible gasotransmitter in the brain. Ammonia can regulate mitochondrial function, membrane sodium potassium ATPase activity and immunity. It plays a role in the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration. Ammonia and thiocyanate serves the purpose of cellular/neuroimmuneendocrine regulation. The archaea can utilize ammonia oxidation for energetics.

Key words: Actinides; Archaea; Urease; Cholesterol oxidase; Ammonia; Thiocyanate

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INTRODUCTION

Actinidic archaea has been related to the pathogenesis of schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration. Actinidic archaea use cholesterol as a carbon and energy source[1-9]. Archaeal cholesterol catabolism mediated by archaeal cholesterol oxidase can produce cholesterol ring oxidation to generate pyruvate. Pyruvate is converted to glutamate by the enzyme serum glutamate pyruvate transaminase. The glutamate gets acted upon by glutamate dehydrogenase to generate ammonia. Archaeal urease can act upon urea generating ammonia and thiocyanate. Ammonia and thiocyanate serves the purpose of cellular and neuroimmune endocrine regulation. The archaea are ammonia oxidizing and can use ammonia for their energetics. The archaeal urease activity related ammonia and thiocyanate synthesis as well as cholesterol oxidase activity generating pyruvate and ammonia was studied in schizophrenia, malignancy, metabolic syndrome x, autoimmune disease and neuronal degeneration.

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+ciprofloxacine 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^{o}C for 1 hour. The following estimations were carried out:- Cytochrome F420, hydrogen peroxide, pyruvate, ammonia, glutamate, thiocyanate and urease activity[11-13] Cytochrome F420 was estimated flourimetrically (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 casued 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 1-4 as percentage change in the parameters after 1 hour incubation as compared to the values at zero time. The results show increased archaeal urease activity generating ammonia and thiocyanate in the disease states. It also shows increased cholesterol ring oxidase activity generating pyruvate. The pyruvate is converted by SGPT to glutamate. Glutamate dehydrogenase converts glutamate to ammonia. There is generation of ammonia by archaeal urease and cholesterol oxidase activity.

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 P value < 0.001		F value 130.054 P value < 0.001	

Table 2
Effect of Rutile and Antibiotics on Pyruvate and Glutamate

Group	Pyruvate % change (Increase with Rutile)		Pyruvate % change (Decrease with Doxy+Cipro)		Glutamate (Increase with Rutile)		Glutamate (Decrease with Doxy+Cipro)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
	4.34	0.21	18.43	0.82	4.21	0.16	18.56	0.76
Schizo	20.99	1.46	61.23	9.73	23.01	2.61	65.87	5.27
Seizure	20.94	1.54	62.76	8.52	23.33	1.79	62.50	5.56
AD	22.63	0.88	56.40	8.59	22.96	2.12	65.11	5.91
MS	21.59	1.23	60.28	9.22	22.81	1.91	63.47	5.81
NHL	21.19	1.61	58.57	7.47	22.53	2.41	64.29	5.44
DM	20.67	1.38	58.75	8.12	23.23	1.88	65.11	5.14
AIDS	21.21	2.36	58.73	8.10	21.11	2.25	64.20	5.38
CJD	21.07	1.79	63.90	7.13	22.47	2.17	65.97	4.62
Autism	21.91	1.71	58.45	6.66	22.88	1.87	65.45	5.08
EMF	22.29	2.05	62.37	5.05	21.66	1.94	67.03	5.97
	F value 321.255		F value 115.242		F value 292.065		F value 317.966	
	P value < 0.001		P value < 0.001		P value < 0.001		P value < 0.001	

Table 3
Effect of Rutile and Antibiotics on Hydrogen Peroxide and Ammonia

Group	H_2O_2 % (Increase with Rutile)		H_2O_2 % (Decrease with Doxy+Cipro)		Ammonia % (Increase with Rutile)		Ammonia % (Decrease with Doxy+Cipro)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.43	0.19	18.13	0.63	4.40	0.10	18.48	0.39
Schizo	22.50	1.66	60.21	7.42	22.52	1.90	66.39	4.20
Seizure	23.81	1.19	61.08	7.38	22.83	1.90	67.23	3.45
AD	22.65	2.48	60.19	6.98	23.67	1.68	66.50	3.58
MS	21.14	1.20	60.53	4.70	22.38	1.79	67.10	3.82
NHL	23.35	1.76	59.17	3.33	23.34	1.75	66.80	3.43
DM	23.27	1.53	58.91	6.09	22.87	1.84	66.31	3.68
AIDS	23.32	1.71	63.15	7.62	23.45	1.79	66.32	3.63
CJD	22.86	1.91	63.66	6.88	23.17	1.88	68.53	2.65
Autism	23.52	1.49	63.24	7.36	23.20	1.57	66.65	4.26
EMF	23.29	1.67	60.52	5.38	22.29	2.05	61.91	7.56
	F value 380.721		F value 171.228		F value 372.716		F value 556.411	
	P value < 0.001		P value < 0.001		P value < 0.001		P value < 0.001	

Table 4
Effect of Rutile and Antibiotics on Archaeal Urease and Thiocyanate

Group	Thiocyanate % (Increase with Rutile)		Thiocyanate % (Decrease with Doxy+Cipro)		Archaeal urease (Increase with Rutile)		Archaeal urease (Decrease with Doxy+Cipro)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Normal	4.40	0.10	18.48	0.39	4.45	0.14	18.25	0.72
Schizo	22.52	1.90	66.39	4.20	23.01	1.69	59.49	4.30
Seizure	22.83	1.90	67.23	3.45	22.67	2.29	57.69	5.29
AD	23.67	1.68	66.50	3.58	23.26	1.53	60.91	7.59
MS	22.38	1.79	67.10	3.82	22.83	1.78	59.84	7.62
NHL	23.34	1.75	66.80	3.43	22.84	1.42	66.07	3.78
DM	22.87	1.84	66.31	3.68	23.40	1.55	65.77	5.27
AIDS	23.45	1.79	66.32	3.63	23.23	1.97	65.89	5.05
CJD	23.17	1.88	68.53	2.65	23.46	1.91	61.56	4.61
Autism	23.20	1.57	66.65	4.26	22.61	1.42	64.48	6.90
EMF	22.29	2.05	61.91	7.56	23.73	1.38	65.20	6.20
	F value 372.716		F value 556.411		F value 391.318		F value 257.996	
	P value < 0.001		P value < 0.001		P value < 0.001		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[14-16]. 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]. The archaeal cholesterol oxidase activity was increased resulting in generation of pyruvate and hydrogen peroxide[14-16]. The pyruvate gets converted to glutamate by serum glutamate pyruvate transaminase. Glutamate is acted upon by glutamate dehydrogenase generating alpha ketoglutarate and ammonia. The archaeal urease acts upon urea as the substrate and generates thiocyanate and ammonia. The archaeal urease and cholesterol oxidase are actinide dependent and activated by rutile. They are suppressed by antibiotics. Ammonia and thiocyanate serves the purpose of cellular and neuroimmune-endocrine regulation. The archaea are ammonia oxidizing and can use ammonia for their energetics.

The archaeal ammonia can regulate brain function. The ammonia can function as a synaptic gasotransmitter. Ammonia can stimulate GABA receptors at high levels and NMDA receptors at low levels. Thus ammonia has a biphasic action in that it can modulate both GABA and NMDA receptors. Thus ammonia can regulate the NMDA/GABA thalamocorticothalamic pathway mediating conscious perception. Ammonia is involved in the pathogenesis of schizophrenia and mood disorders. Ammonia can stimulate membrane sodium potassium ATPase activity. Membrane sodium potassium ATPase when stimulated leads to decrease in intracellular calcium and increase in intracellular magnesium resulting in modulation of multiple neurotransmitter systems. The neurotransmitter release from the presynaptic vesicles is calcium dependent[17-20].

Membrane sodium potassium ATPase uses 80 percent of the mitochondrial synthesized ATP. Elevated ammonia levels results in a hyperactive membrane sodium potassium ATPase and exhausts all ATP reserves. The mitochondria get fatigued leading on to mitochondrial dysfunction. Ammonia can open the mitochondrial permeability transient, produce cytochrome C release and activate the caspase cascade. Cyanide ion released by urease action can produce cytochrome C oxidase inhibition and mitochondrial dysfunction. Mitochondrial dysfunction can lead onto neuoronal degeneration[17-20].

Ammonia inhibits insulin release from beta cells contributing to the diabetic state. The hyperactive membrane sodium potassium ATPase due to increased ammonia levels produces increased ATP usage and mitochondrial fatigue. The mitochondrial PT pore opening produce by ammonia and related mitochondrial dysfunction leads onto inefficient energetics and metabolic syndrome x. The archaeal urease activity generates the thiocyanate ion from urea. Cyanide inhibits

mitochondrial cytochrome C oxidase and produces mitochondrial dysfunction. Cyanide toxicity has been related to mucoid angiopathy implicated in coronary artery disease and strokes. Cyanide toxicity leads to pancreatic dysfunction and diabetes mellitus manifesting as chronic calcific pancreatitis. Cyanide toxicity can also lead to multinodular goiter and endomyocardial fibrosis. Thus the generation of thiocyanate from urea by the activity of archaeal urease can contribute to a cardiac endocrine syndrome. Thiocyanate can also modulate protein function and structure by binding to proteins. This produces thiocynalation of proteins. Thus thiocyanate can modulate cell function[18-23]. Ammonia can function as a mutagen and contribute to alteration in DNA function.

Ammonia can also modulate immune function. It can alter Tcell and B cell function. Ammonia can be immunostimulatory or immunosuppressive depending on its levels. Ammonia can contribute to the genesis of autoimmune disease[24-26]. Ammonia can also produce cell proliferation and oncogenic transformation as exemplified in gastric carcinoma and hepatomas. During autophagy, portions of the cytoplasm are sequestered into autophagosomes and digested by lysosomal hydrolases. Massive autophagy can be induced in mammalian tissues in a coordinated fashion through nutrient deprivation, which has prompted the search of soluble metabolites that can stimulate autophagy. Ammonia, which is generated as a by-product of glutaminolysis, has been identified as a diffusible factor that stimulates autophagy. Intriguingly, cancer cells increase the rate glutaminolysis and the interstitial fluid of cancers contains higher-than-normal physiological concentrations of ammonia, suggesting a previously unknown pathway through which tumor cells can condition their microenvironment[27,28].

Thus ammonia and thiocyanate produced by endosymbiotic archaeal metabolism modulate neural transmission, metabolic/mitochondrial function, immunity, cell death, cell proliferation and endocrine/pancreatic function. Thus the archaea can use ammonia as a signaling molecule to produce neuro-immune-metabolic-endocrine integration. The archaea are ammonia oxidizing and can use ammonia for their energetics[29] The archaeal utilization of ammonia as a signaling molecule and for energetics may be a remnant of ammonia based primitive archaeal life forms and metabolism. Liquid ammonia can replace water as a solvent for life to originate.

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