D-Amino Acids are Signaling Agents Under Stress, that Broadly Impact Preventive Medicine

Edna Ben-Izhak Monselise

Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel,
bened@post.bgu.ac.il

Dedication:

This article is dedicated to my dear husband and best friend, Moshe Melech Ben-Izhak who, has been courageously withstanding ALS disease since March18th 2018 and inspiring, everyone around him.

Key Words:
D-Glutamate and D-Glutamate racemase; Mitochondria; evolutionary approach; gut microbiota; Amyotrophic Lateral Sclerosis (ALS); Motor neurone disease (MND)

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Abstract

Three different fields intersect in search of an understanding of the point-of-origin of modern-age diseases: 1) D-amino acids and their role under stress conditions; 2) evolutionary origin of the mitochondrion organelle in the eukaryotic cell; and 3) gut microbiota and human health

Here it is first suggested that **D**-amino acids function as **universal signaling agents**, after having evolved as prokaryotic communication, part of an organic communication process that governs the basic activities of all the cells and coordinates cell action.

Mitochondria (symbiotic prokaryotic organelles), are creative source of D-amino acids as signaling agents in the central nervous system and in the neuroendocrine systems.

Amino acids racemases catalyzes the conversion between the L-enantiomers (protein building blocks) into D-enantiomers (signaling agents).

It is suggested that hectic modern life may affect human health by causing stress to the gut microbiota. These affected, gut microbiota then secrete **D**-amino acids that enter the blood stream, as signaling agents, causing communication errors in the central nervous system, and in the neuroendocrine systems due to excessive quantity of D-amino acids.

Treating gut microbiota with inhibitors of amino acids racemases or finding D-amino acid scavengers may be used in developing novel therapeutic strategies for diseases related to the central nervous system and neuroendocrine systems caused by stressed gut microbiota.

1) D-amino acids and their role under stress conditions

Amino acids have an α-carbon that is connected to four functional groups: an amine group ($-NH_2$), a carboxyl group (-COOH), a hydrogen (-H) and a side chain (-R). Therefore, looking at the chemical formula of amino acid is insufficient, since it does not take into consideration, three dimensional arrangement of the molecule as L-enantiomers or D-enantiomers. These stereoisomers are not superimposable mirror images to each other.

I have been studying stress on the biochemical and biophysical levels. My cell culture were duckweed plants grown in darkness, for 5 months, causing them to lose all their natural protection (chlorophyll and the potential of creating free-radical scavenger-vitamin C) they served as my models towards reaching a general understanding of first cellular distress signals.

Alanine became the first organic distress signal under many abiotic conditions [1-5].

Only recently, I realized that this distress signal was more sophisticated, as it was **D**-alanine and not L- alanine [6].

The majority of amino acids in higher animals were thought to be L-enantiomers while D-amino acid enantiomers were considered unnatural. D-amino acids are indispensable for bacterial growth as components of cell wall peptidoglycans. However, advanced analytical techniques that detect chiral amino acids [6-11] have demonstrated that several D-amino acids are present in mammals, including humans. [12, 13]. Moreover, physiologic functions of several D-amino acids have been identified to date. In particular, D-serine regulates nervous signaling in the cerebral cortex and participates in memorization and learning. D-Aspartate is often present in the central nervous system (CNS), and neuroendocrine, and endocrine systems and plays physiological roles in the regulation of hormone secretion and steroidogenesis [14-16]. Table 1 presents 24 examples of D-amino acids accumulation, as signaling agents, under various stress or disease conditions: this a universal phenomenon in prokaryotic and eukaryotic cells.

D-Alanine

D-Alanine is detected in the brain, pituitary gland, pancreas, adrenal gland, and testis of rodents [17]Moreover, it is also detected in the human brain.[18]Most of the D-alanine in rodents is derived from intestinal bacteria[19,20].

D-alanine is metabolized by D-amino-acid oxidase (DAO) [21]and the levels of D-alanine in rodents depend on their circadian rhythm.

[22,23] Circadian changes in D-alanine amounts were observed in the urine and serum of humans. These 24-h profiles of D-alanine are almost the same as those observed in rats and mice, suggesting that D-alanine has fundamental physiological functions related to rest/active conditions in mammals [24].

In addition, it has been reported that D-alanine binds to the N-Methyl-D-aspartic acid-receptor (NMDA receptor) and alleviates symptoms in schizophrenia patients [25,26].

D- Glutamate

D-glutamate is an essential, biosynthetic building-block for all gram-positive and gram-negative bacteria. It is incorporated into the peptidoglycan monomeric unit by the MurD enzyme, and is necessary for the successful production of the peptidoglycan (murein) component of bacterial cell walls[27].

Additionally, D-glutamate is the primary constituent of the *Bacillus anthracis* poly-γ-D-glutamyl capsule (one of the two virulence factors of the disease anthrax) and functions to protect the organism against the bactericidal components of serum and phagocytic engulfment[28].

Prior studies of exogenous D-glutamate suggest that it is metabolized by dietary enzymes and bacterial flora, [29]as well as being metabolized by the engulfed organelles mitichondria [30,31](as per the ancient endosymbiotic relationship). D-glutamate is found in the rodent brain [32,33].

The enzyme glutamate racemase (RacE) appears to be the primary source of D-glutamate for cell wall biosynthesis, and is also unique to bacteria, making it a potentially attractive target for antimicrobial drug-design[34].

D-Glutamate is metabolized in eukaryotic cells within the mitochondria and chloroplast[31 35, 36].

The amino-acid glutamate, synthesized in the mitochondria, serves multiple functions, including acting as a neurotransmitter and participating in degradative and synthetic pathways, depending, which enantiomer: L or D.

Moreover, studies on the enzymes that synthesize or metabolize D-amino acids have also clarified the localization and functions of D-amino acids in the CNS and endocrine systems, and the physiological functions of these D-amino acids are being gradually becoming known. It has been demonstrated that D-amino acids, such as D-serine, D-aspartate, D-alanine, and D-cysteine, play important roles in the CNS and endocrine systems. Therefore, it is very important that the mechanisms of synthesis and metabolism as well as the physiological functions of D-amino acids are investigated further. These investigations will provide new therapeutic and diagnostic strategies for diseases related to the nervous and endocrine systems.

Glutamate racemase

Glutamate racemase is an enzyme that catalyzes the stereochemical inversion around the asymmetric carbon atom in a substrates having only one center of asymmetry.[37] Glutamate racemase is a member of a rare family of cofactor-independent racemases and epimerases[38,39].

including aspartate racemase, proline racemase and diaminopimelate epimerase. Extensive mechanistic studies on glutamate racemase from *Lactobacillus fermenti* demonstrated that racemization of glutamate proceeds via a deprotonation/reprotonation mechanism similar to that of alanine racemase.[40414243]

A primary sequence homology was found in the glutamate racemase of *Bacillus sphaericus* ,glutamate racemases from other bacteria, and the putative gene products of *Bacillus subtilis racE* and *yrpC* genes.

In eukaryotic mammalian cells, D-glutamate was found to be metabolized in the mitochondria catalyzed by the enzyme glutamate racemase[44, 45].

2) Evolutionary origin of the mitochondrion organelle in the eukaryotic cell

Mitochondria and chloroplasts likely evolved from engulfed bacteria that had once lived as independent organisms. At some point, an eukaryotic cell engulfed an aerobic bacterium, which then formed an endosymbiotic relationship with the host eukaryote, gradually developing into a mitochondrion[46]. A dominant role of the mitochondria is the production of adenosine tri-phosphate (ATP) by means of aerobic respiration, dependent on the presence of oxygen[47-50].

Mitochondria have their own independent genomes that shows substantial similarity to bacterial genomes [46, 51-56].

Mitochondria are essential for ensuring numerous fundamental physiological processes such as cellular energy, redox balance, modulation of Ca^{2+} signaling[57-59]and important biosynthetic pathways. They also govern the cell fate by participating in the apoptosis pathway[60].
The regulation of these parameters has an impact on mitochondrial function, especially in the central nervous system. The amino-acid D-glutamate is synthesized in the mitochondria and acts as a neurotransmitter[36].

3) Gut microbioa and human health

More than 90% of the human body consists of non-human cells. The various microbiota colonizing our skin, intestines, respiratory tract, mouth, and urogenital tract, consists of bacteria, viruses, parasites, and fungi whose genes collectively outnumber the quantity of human genes by a factor of 100. For a long time, it was thought that these microorganisms are mostly passive members of the human ecosystem, primarily involved in our intestinal digestive functions. It is now clear, however, that the members of the microbiota are an integral part of human physiology. This microbial presence and activity influences the function of the immune system, the CNS and the metabolic system, as well as impacting organ development. In addition, microbial colonization affects a large variety of disease processes, ranging from chronic inflammatory disease to autoimmunity, obesity, and cancer[61-65].As such, the microbiota should be considered as yet another human system, comprising a multitude of cells, genes, and metabolic pathways, that performs pivotal functions in both human health and disease[66].

Interactions between mammalian hosts and their microbial counterparts.

The human meta-organism has evolved as a unity of both eukaryotic and prokaryotic

cells, and we are exploring the mechanisms by which this co-evolution has lead to stable community formation and homeostatic host-microbial mutualism .
Emerging evidence has demonstrated that the gut microbiome plays essential roles in the pathogenesis of human diseases in distal organs[67, 68].

Multidirectional interactions between the CNS and immune systems have been documented in homeostasis and pathologies ranging from leukemia to acute and chronic inflammation.[69]and from multiple sclerosis to autism spectrum disorder (ASD) [70]. The gut microbiota regulates behaviors in mice via the production of neuroactive metabolites, suggesting that gut-brain connections contribute to the pathophysiology of ASD.

Environmentally-driven microbiome-brain interactions may modulate murine ALS, and call for similar investigations in human ALS[71].

A potential role for D-amino acids in motor neuron disease/amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease (MND), is a progressive neurodegenerative disease of the motor neurons, resulting in the gradual weakness of the voluntary muscles until death from respiratory failure occurs after about three years[72, 73].
A potential role for D-amino acids in MND/ALS is emerging. D-serine, which is an activator/co-agonist at the N-methyl-D-aspartate glutamate receptor subtype, is elevated both in the spinal cord (from sporadic cases of ALS) and in an animal model of ALS [74]. A pathogenic mutation in DAO, is associated with familial ALS that impairs D-serine metabolism and causes protein aggregation, autophagy and cell death in motor neuron cell lines[75].
Although great advances have been made in the understanding of the genetic causes of ALS, the contribution of environmental factors preceding the onset of ALS, has also been studied, but has not yet revealed a replicable, definitive environmental risk factor[76].
Many of these damaged functions are regulated by signalling between the endoplasmic reticulum and the mitochondria, which has stimulated further investigations into the roles of the endoplasmic reticulum, and mitochondria signaling[35,77, 78].

Rilutek is used to treat ALS. Riluzole helps to slow course of this degenerative disease and prolong survival, although, it is not a cure, and does not reverse the nerve damage or muscle weakness. Riluzole is thought to work by protecting the nerves in the brain and spinal cord from **an excess, of D-glutamate** that may be a partial cause of nerve damage[79,80].

Inhibition of glutamate racemase

D-glutamate is an essential biosynthetic building block of the peptidoglycans that encapsulates the bacterial cell wall. Glutamate racemase catalyzes the reversible formation of D-glutamate from L-glutamate and, hence, the enzyme is a potential therapeutic target [44, 81-89].

Summation

I have presented a novel interpretation of the data by combining them in a very simple and uncomplicated way to explain the impact of modern-day stress on the neurobiochemical signaling in the human body, that may consequently cause ill-health due to internal systemic miscommunicantions.

My theory links eight important facts:

1. D-amino acids exist in human -eukaryotic cells.
2. D-amino acids play a role as signaling agents in the CNS, the neuroendocrine , and endocrine systems, and in the regulation of hormone secretion.
3. D-amino acids are created by the organelles mitochondria.
4. Mitochondria evolved from engulfed prokaryotic cells (an aerobic bacteria that once lived as independent organisms).
5. D-amino acids are created under stress either in mitochondria or in prokaryotic cells.

6. The microbiota may be considered as forming an additional human system, comprising a multitude of cells, genes, and metabolic pathways, that performs pivotal functions in both human health and disease.
7. D-amino acids created by gut microbiota may affect signaling agents in the CNS, neuroendocrine and endocrine systems, causng excessive quantity of D-amino acids.

8. Since racemases catalyzes the reversible formation of D-amino acid from L-amino acid, controling these enzymes may provide therapeutic solutions.

Hectic modern lifestyle may affect human health by causing stress to the gut microbiota, thus setting off a neurobiochemical chain reaction. As a result, the gut microbiota secrete their signaling agents, D-amino acids, as their distress beacons. Then these affected D-amino acids, travel via the blood stream throughout the entire circulatory system disrupting the signaling in CNS, neuroendocrine, and endocrine systems, and in the regulation of hormone secretion. This disruption may occur as the nerve cells also use the same archaic means of communication- the D-amino acids, secreted by their own mitochondria.
Fig.1 presents what may happen in, Motor Neurone Disease (MND). D-glutamate created by gut microbiota may affect signaling agents in the CNS, causing excessive quantity of D-glutamate at the chemical synapses.

I have suggested **a new approach** – trying to locate and understand the source of human neurobiochemical miscommunication.
Having discovered the impact of daily stress on the universal role of the D-amino acids. I suggest that seeking ways to prevent or correct such miscommunication may well lead to the prevention or curing of many modern-age diseases.
As such, there is need of much more research on D-amino acids racemases in the human microbiom and in mitochondria and of a search for these enzymes inhibitors, the results of future studies may produce new therapeutic strategies for preventing and combating diseases that affect the CNS and/or endocrine systems.

Acknowledgements:

I wish to thank Prof. Daniel Kost and Aliza Levkovitz, for the ^{15}N- NMR spectrometric research work done together for the last 30 years.
I wish to thank Prof. Dudy Bar-Zvi for critically reading the manuscript.
Many thanks to Dorit van-Moppes Library Information Specialist.
Many thanks to my English editor, Ethelea Katzenell.

Figure 1: Illustrating the connection between a stressed gut microbiota releasing D-glutamte into the blood stream as a communication signal, and the resulting impact on motor neurons

It presents what may happen in, Motor Neurone Disease (MND). D-glutamate created by gut microbiota may affect signaling agents in the CNS, causing excessive quantity of D-glutamate at the chemical synapses.

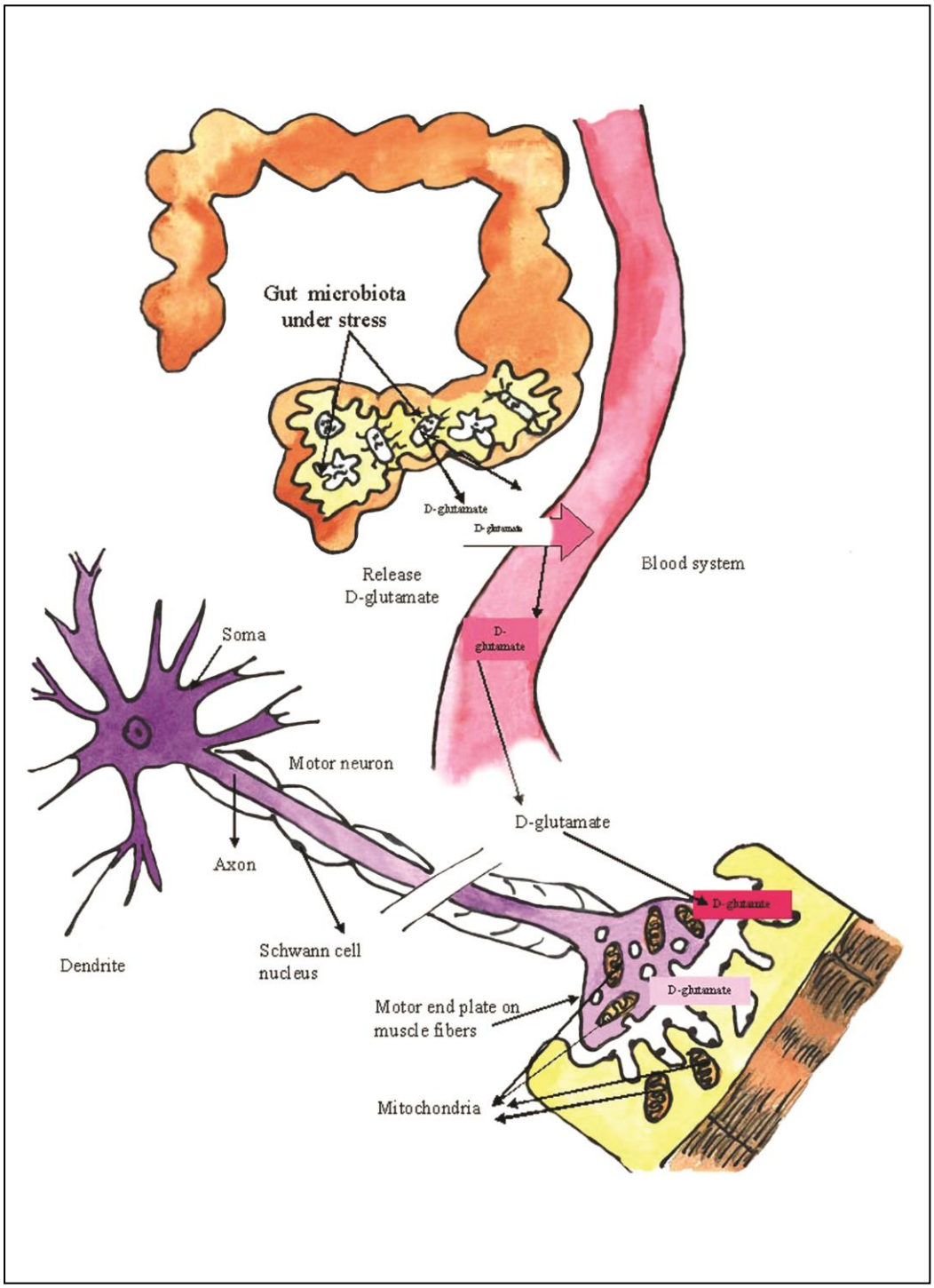

Table 1: D-amino acid accumulation, as signaling agents, under various stress or disease conditions- a universal phenomenon

Entry No.	Type of stress/ disease	organism	The D-amino acid accumulated	Ref
		prokaryotic		
1.	In Vivo Infections early stages of disease interaction with murine macrophages	Bacteria *Bacillus anthracis*	D-alanine	90
2.	Hyperthermal stress	Archaea bacteria *Pyrobaculum islandicum Methanosarcina barkeri Halobacterium salinarium*	D-alanine	91
3.	A mutant in *Vibrio cholerae* mrcA	Bacteria a mutant in Vibrio cholerae mrcA, which encodes a PBP1A homolog and *Bacillus subtilisgenerated*	D-Met and D-Leu D-Tyr and D-Phe	92
		eukaryotic		
4.	Osmotic stress	Parasitic protozoan *Leishmania amazonensis*	D-alanine	93
5.	Hypersalinity acclimation	Crustaceans Aquatic invertebrates *Penaeus japonicus Procambarus clarkia Juasus lalandi Chionoecetes opili* Eriocheir japonicus	D-alanine	94
6.	Changes in external salinity	A brackish-water mollusc, *Corbicula japonica*	D-alanine	95
7.	Hypersalinity acclimation	Mollusks Aquatic invertebrates *Scapharca broughtonii Crassostrea gigas Patinopecten yessoensis Meretrix lusoria Ruditapes philippinarum Pseudocardium sachalinensis Tresus keenae*	D-alanine	94
8.	Hypertonic or Hypotonic stress	Mollusks aquatic invertebrates *Lucinoma aequizonata*	D-alanine	96
9.	Herbicides	Plant *Nicotiana tabacum*	D-alanine	97
10.	Ultraviolet radiation	Duckweed plants *Landoltia punctata*	D-alanine	6
11.	Amino acid deprivation	Plant *Arabidopsis thaliana*	D-alanine	98

Entry No.	Type of stress/ disease	Organism	The D-amino acid accumulated	Ref
12.	Tidal freshwater marshes	Plant *Phragmites australis*	D-alanine	99
13.	Most exposed to chronic mild stress (CMS), also some of themwith Alzheier's disease (AD)	Male Wistar Rats mammalian tissues frontal cortex	D-glutamate	100
14.	Mutant lacing D-amino acid oxidase	Mouse mammalian tissues	D- amino acids: D- serine ; D-alanine; D- proline	101 -104
15.	Treated with vehicle or drugs employed for therapy of mood/anxiety and subjected to food shock stress	Rats mammalian tissues	D-glutamate	105
16.	Adult male	Rat mammalian tissues salivary glands	D-aspartic acid	106
17.	Adult male	Rat mammalian tissues CNS anterior pituitary gland and in the pancreas	D-alanine	22
18.	Mutant lacing D-amino acid oxidase	Mouse mammalian tissues	D- praline; D- leucine	107
19.	Mutant ddY/DAO^{-} mice lacking D-amino-acid oxidase	Mouse mammalian tissues in the pituitary and pineal glands	five D-amino acids (D-Asp, D-Ser, D-Ala, D-Leu and D-Pro)	108
20.	Adult male	Rat mammalian tissues Islets of Langerhans of rat pancreas	D-alanine	109
21.	Renal - Kidney disease-	Human *Homo sapiens* mammalian tissues	D- amino acids: D- serine ; D-alanine; D- proline	110
22.	Hunger	Human *Homo sapiens* mammalian tissues produced in salivary glands	; D-alanine; D-proline; D-aspartate	111
23.	Alzheimer's disease (AD)	Human	D-aspartate	112
24.	Motor Neuron Disease(MND)/Amyotrophic Lateral Sclerosis(ALS)	Human	D- serine; D-glutamate; D-aspartate	74

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