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Neurochemical Imbalances and Autism:
Tetrahydrobiopterin, Tryptophan, Serotonin Issues and Quinolinic Acid Toxicity

Support Documents for Module #12

- Ibuprofen Dosing Instructions - *handout*
- Lecture slides *(pdf)*
- Lecture slides note taking *(pdf)*
- Lecture slides – *Phospholipase A2 (pdf)*
- Lecture slides – *Phospholipase A2 note taking (pdf).*

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Lecture Overview

- Methylation and Biopterin metabolism
- Tetrahydrobiopterin (BH4) and its connection to dopamine, serotonin and tyrosine.
- Tryptophan metabolism and BH4
- The role of Quinolinic Acid (QA):
  - *NMDA stimulation and other adverse effects*
- The *potential* neurotoxic effect of tryptophan supplementation.
- Tryptophan versus 5-HTP supplementation
- Treatment options for high QA

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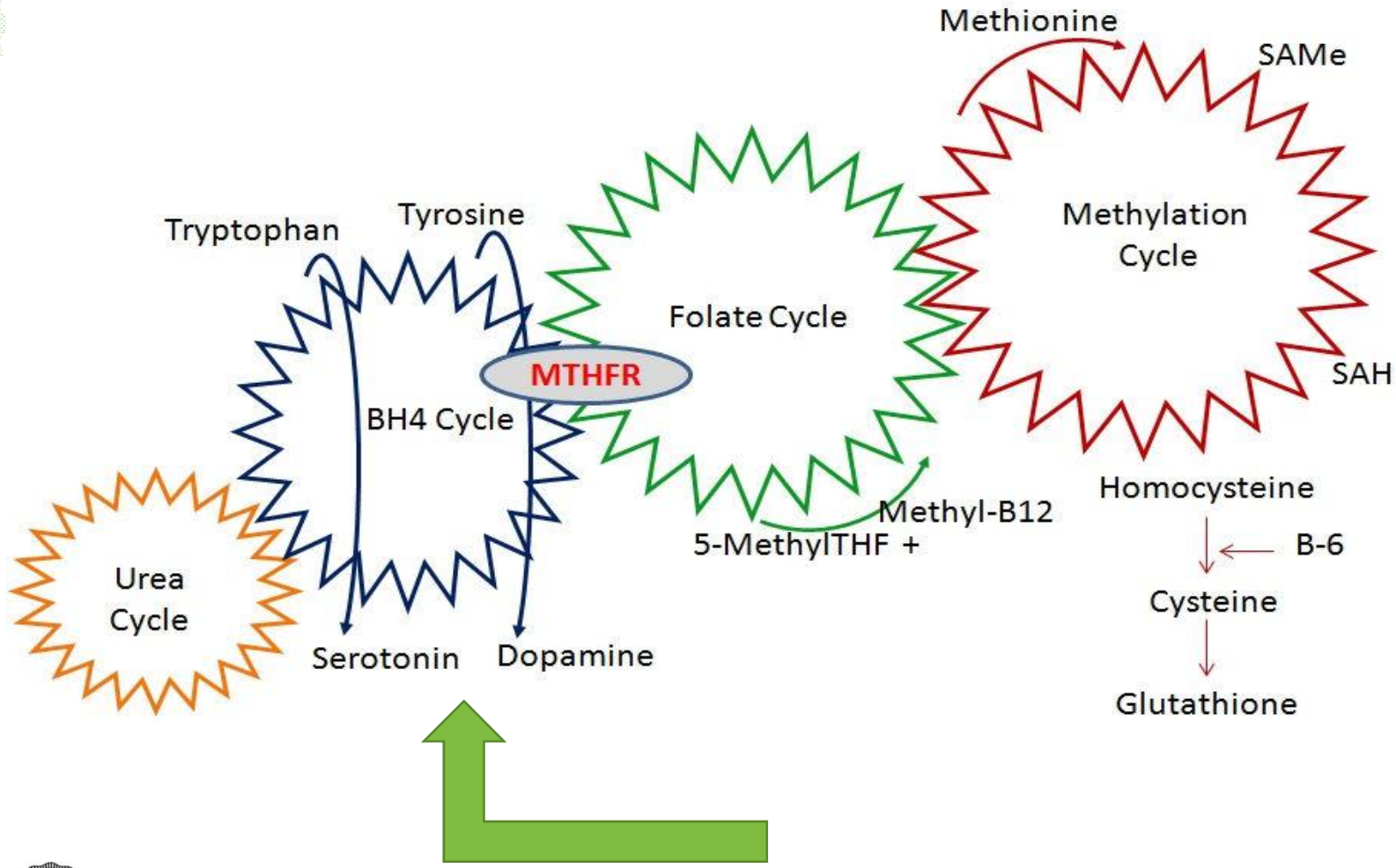
Between The Wheels



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Methylation and Biopterin Metabolism

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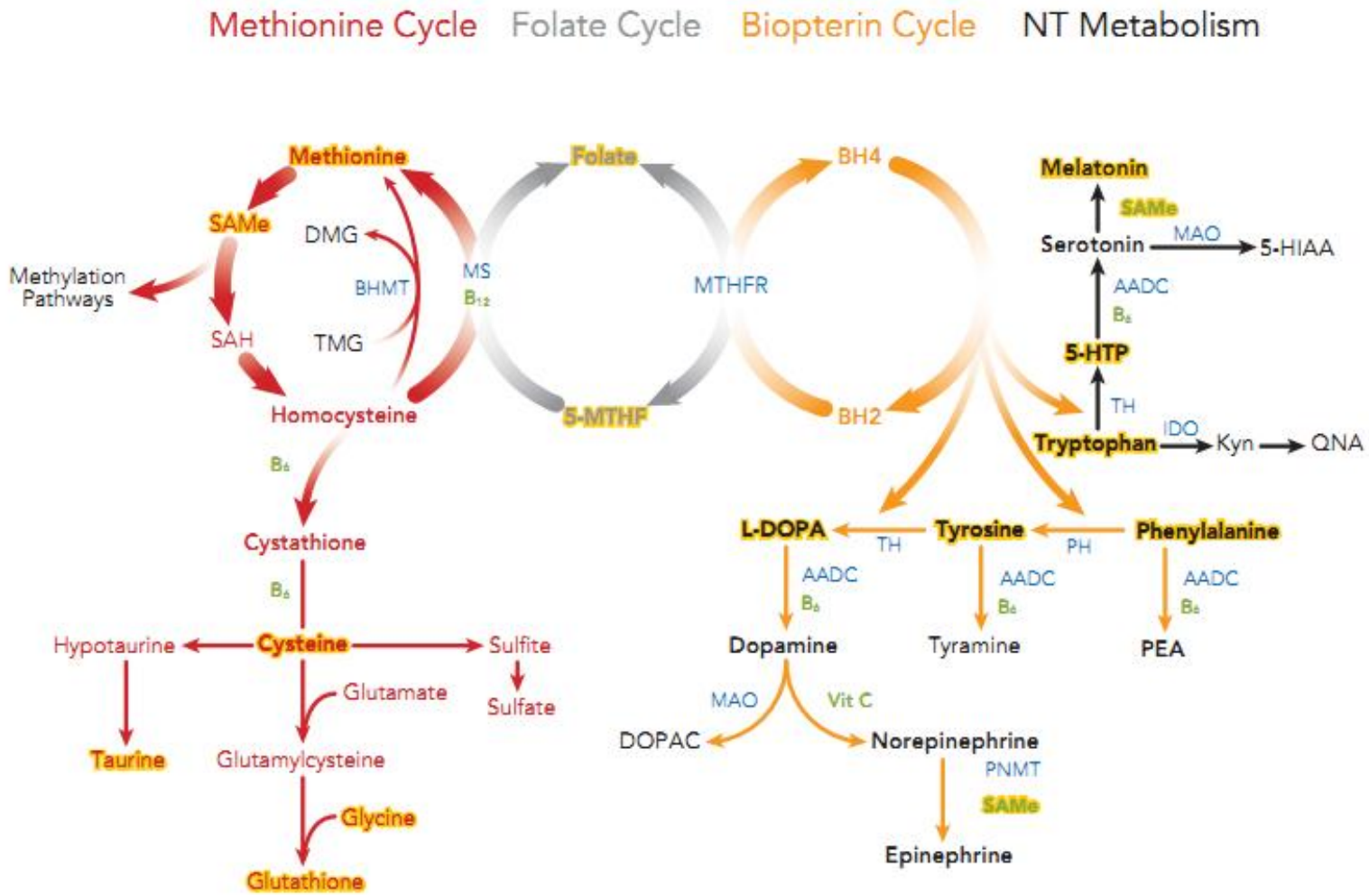
Methylation Biochemistry



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MTHFR gene SNP - *A1298C*

- MTHFR = **M**ethylene**T**etra**H**ydro**F**olate **R**eductase
- Two mutations – **C677T** and **A1298C**
- **677** – *related more to folate metabolism*
- **1298** – *dopamine, serotonin, ammonia, tyrosine & tryptophan metabolism by augmenting BH4:*
  - *Mutation affects the way Methylenetetrahydrofolate reductase uses methyl-folate in producing BH4 (tetrahydrobiopterin).*
- Each can be - -, + -, or + +

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DNA Methylation Pathway Panel – *Doctors Data*

Gene Name / Variation	Not Present	Present	Call
SHMT / C1420T	-/-		G
AHCY / 1	-/-		A
AHCY / 2	-/-		T
AHCY / 19	-/-		A
MTHFR / C677T	-/-		C
MTHFR / A1298C		+/+	
MTHFR / 3	-/-		C
MTR / A2756G	-/-		A
MTRR / A66G		+/-	Hetero
MTRR / H595Y	-/-		C
MTRR / K350A	-/-		A
MTRR / R415T	-/-		C
MTRR / S257T	-/-		T
MTRR / 11		+/-	Hetero
BHMT / 1	-/-		A
BHMT / 2		+/-	Hetero
BHMT / 4		+/-	Hetero
BHMT / 8		+/-	Hetero
CBS / C699T		+/+	T
CBS / A360A	-/-		C
CBS / N212N	-/-		C
COMT / V158M		+/+	A
COMT / H62H		+/+	T
COMT / 61	-/-		G
SUOX / S370S	-/-		C
VDR / Taq1	-/-		C
VDR / Fok1	-/-		C
MAO A / R297R		+/+	T
NOS / D298E		+/-	Hetero

Minus "-" represents no mutation

Plus "+" represents a mutation

"-/-" indicates there is no mutation

MTHFR A1298C

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GPL-SNP 1000 DNA Sequencing Profile

The Great Plains Laboratory, Inc. Health, Metabolism & Nutrition

William Shaw, Ph.D, CLIA Laboratory Director
CLIA ID # 17D0919496

First Name	Last Name	DOB	Sex	Ordering Physician	GPP
Patient 1	GPL Test	1/1/79	Male		GPP23846
Sample Condition	**Sample Type**	**Test Order Date**	**Collection Date**	**Customer Reference**	**Posting Date**
		6/9/15	N/A	N/A	9/14/15

Key

+ + Both copies of the chromosome have the described mutation	Likely benign
+ – One copy of the described mutation exists in this patient	Mutation of unknown significance
– – No mutation exists on either chromosome (see appendix)	Small risk of being pathogenic
	Increased pathogenic risk
	Likely pathogenic

Results

DNA METHYLATION

Mutations: **2**

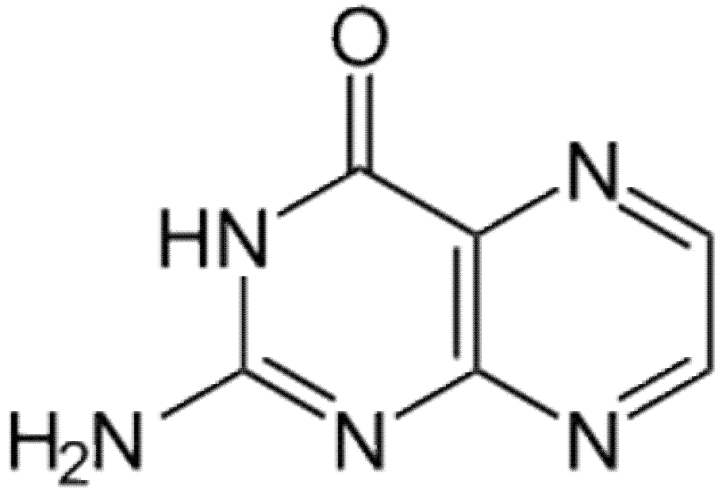
The methylation pathway is the complex process by which carbons are added onto folic acid from various substrates and redistributed onto other compounds in the body, through the use of several enzymes. Methylation is often referred to as one-carbon donation. This pathway is responsible for the formation of methionine (a sulfur based amino acid) and thymidylate monophosphate (dTMP). These compounds play critical roles in nucleotide synthesis, neurotransmitter function, detoxification, and numerous other processes. The recycling of carbons for use in the methylation pathway is critical for cellular function. These enzymes are coded for on the end of the 21st chromosome, making them more susceptible to mutations.

Gene	RS Number	Pathogenicity	Genotype	Phenotype	Disease Associated
CBS	rs2851391		T,C	+ –	Homocysteine levels are altered
VDR	rs1544410		C,T	+ +	Increased risk of osteoporosis

MENTAL HEALTH

Mutations: **2**

Pterin



- Heterocyclic compound
  - *Two different atoms attached to ring structure.*
- Pteridine ring system
  - *Contains Keto & Amino group*
- Folate is a derivative of pterin.
- Pterins are complexed into Biopterins.

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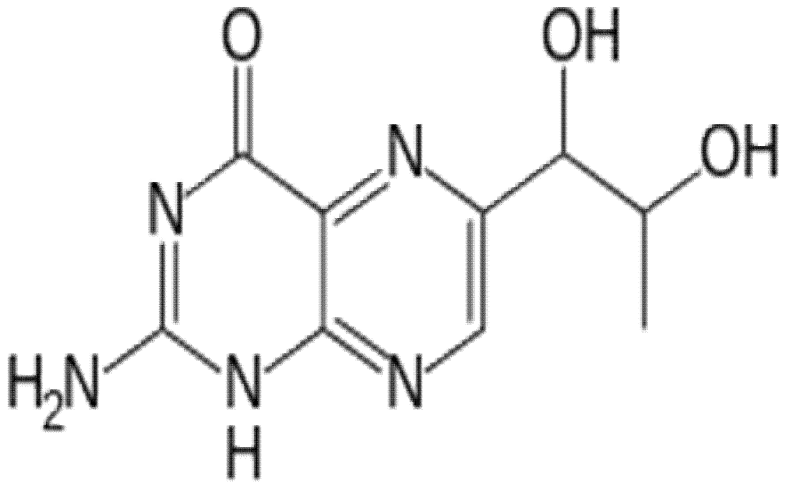
Biopterin

- Acts as cofactor for Aromatic Amino Hydroxylases:
  - *Produce dopamine, serotonin, norepinephrine, and epinephrine.*
  - *Nitric oxide production*
- BH4 (tetrahydrobiopterin) and BH2 (dihydrobiopterin) are the two principle biopterins found in the human body.



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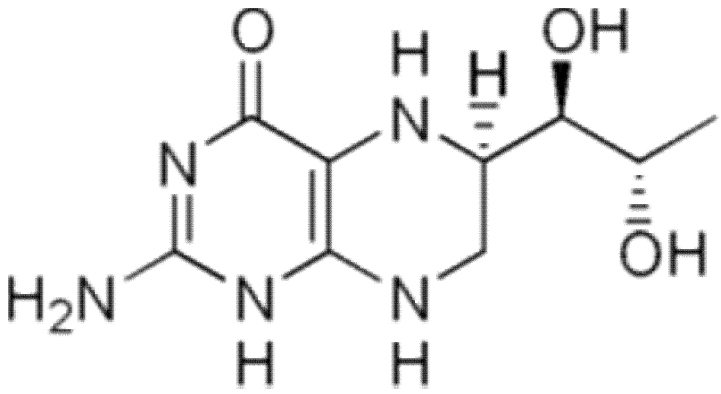
Tetrahydrobiopterin

- Cofactor for Phenylalanine hydroxylase (PAH) to degrade L-Phenylalanine (PHE) to L-Tyrosine (TYR).
- Cofactor for Tryptophan hydroxylase to convert L-Tryptophan (TRP) to 5-Hydroxytryptophan (5-HTP).
- Cofactor for Tyrosine hydroxylase (Th) to convert L-Tyrosine (TYR) to L-DOPA.
- Cofactor for Nitric oxide synthase to convert L-Arginine to Nitric oxide.



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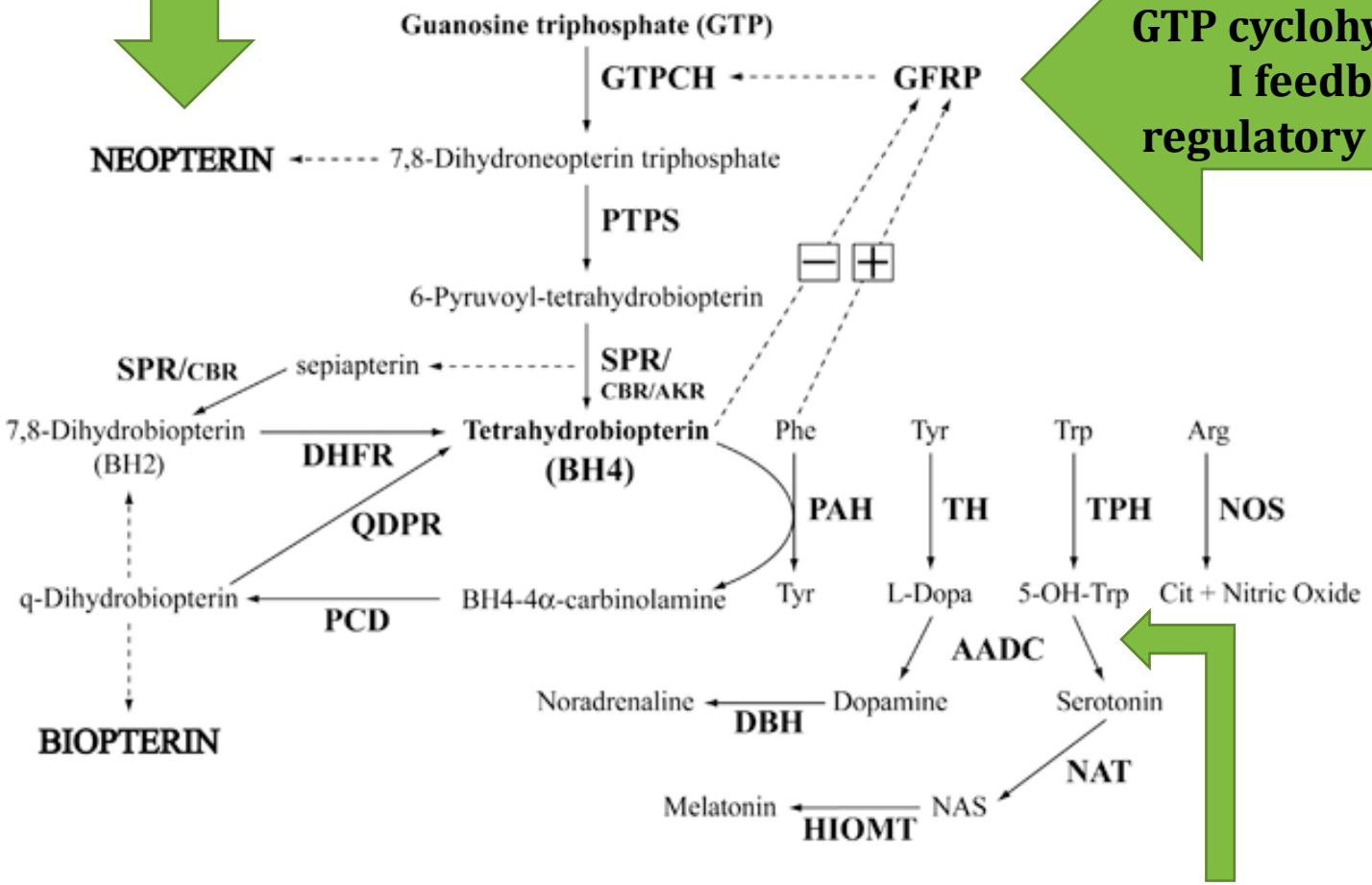
Neopterin produced in response to immune activation



GTP cyclohydrolase I feedback regulatory protein

AADC = aromatic amino acid decarboxylase

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GENOVA DIAGNOSTICS
www.gdx.net • 800.522.4762

Ordering Physician:

John Doe, MD

1234 Main St.
Anywhere, GA 30096

Accession #:	**A1207020275**	Date Collected:	07/01/2012
Order #:	G1234567	Date Received:	07/02/2012
Reference #:		Date of Report:	07/03/2012
Patient:	**Sample Report**		
Date of Birth:	02/05/1962	Telephone:	7704464583
Age:	50	Fax:	7704412237
Sex:	Female		
Reprinted:	07/12/2013		
Comment:			

neopterin biopterin profile

0088 Neopterin/Biopterin Profile - Urine

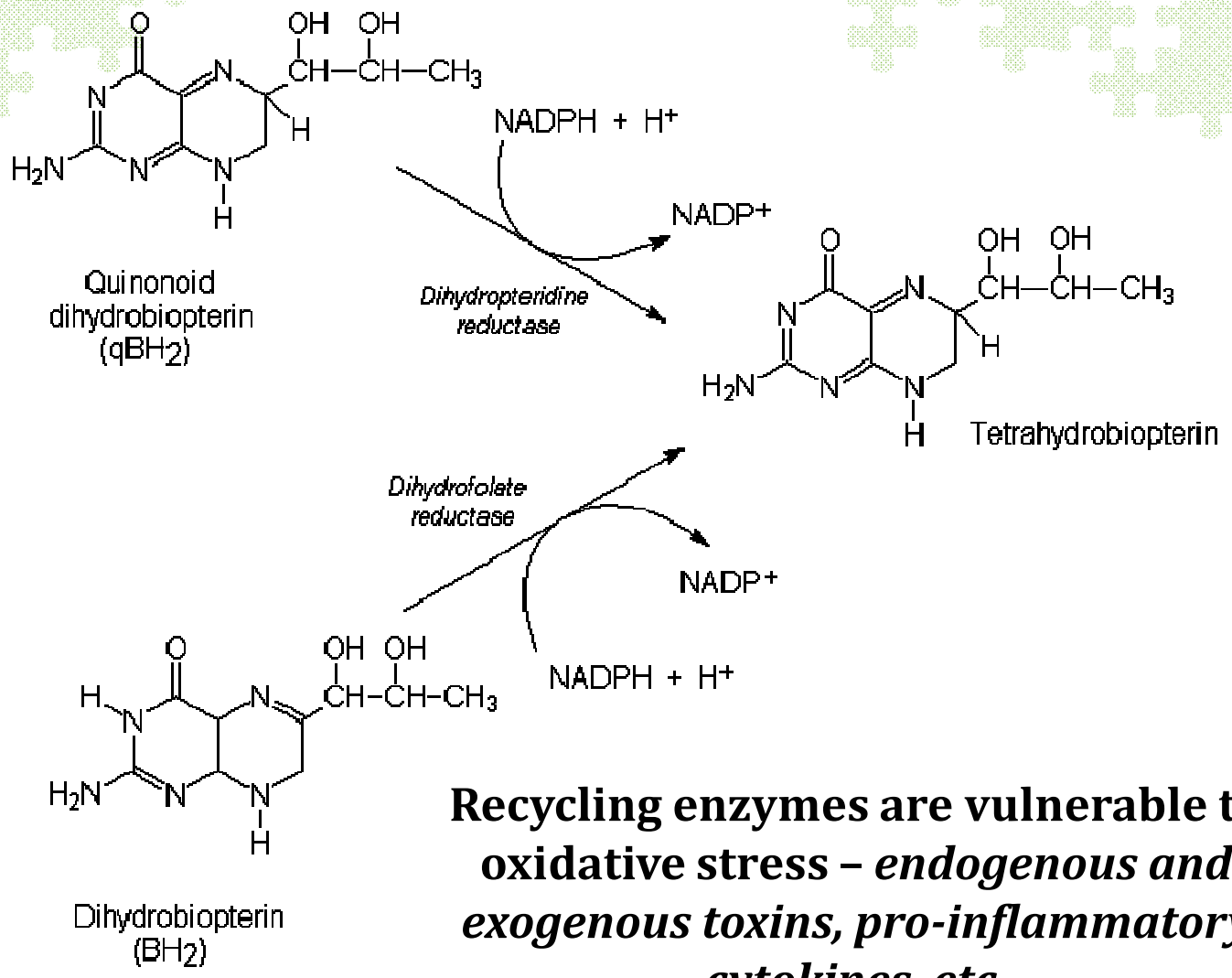
Methodology: LC/Tandem Mass Spectroscopy, Colorimetric

Compound Tested	Results mcg/mg creatinine		Quintile Ranking (1st 2nd 3rd 4th 5th)	95% Reference Range
Ranges are for ages 13 and over				
1. Neopterin	0.55	H	0.18 – 0.53	0.15-0.79
2. Biopterin	0.24		0.05 – 0.26	0.04-0.35
3. Neopterin/Biopterin ratio	2.29		0.78 – 5.02	0.04-8.67
Creatinine = 200 mg/dL				

<DL = less than detection limit

Interpretation:
Neopterin is a marker of inflammatory challenge such as that precipitated by interferon gamma in response to viral infection or intestinal bacterial overgrowth. Urinary neopterin elevation has been proposed as a surrogate marker for inflammatory diseases. Neopterin and biopterin tend to respond similarly except in conditions such as autism where biopterin tends to rise while neopterin falls in CSF. Such scenarios are most sensitively detected by an abnormal neopterin/biopterin ratio. These markers allow assessment of successful strategies to reduce chronic inflammation.

Values in the first decile are reported as 'L' because they may have significance regarding a patient's ability to produce adequate tetrahydrobiopterin (BH4). BH4 is required for the Phe to Tyr conversion and for formation of nitric oxide and serotonin. The method being used for this assay allows accurate low range eterminations that were not possible by earlier methods for neopterin. Patients with insufficient tetrahydrobiopterin synthesis may benefit by supplemental H4 and folate.



Recycling enzymes are vulnerable to oxidative stress – *endogenous and exogenous toxins, pro-inflammatory cytokines, etc*.

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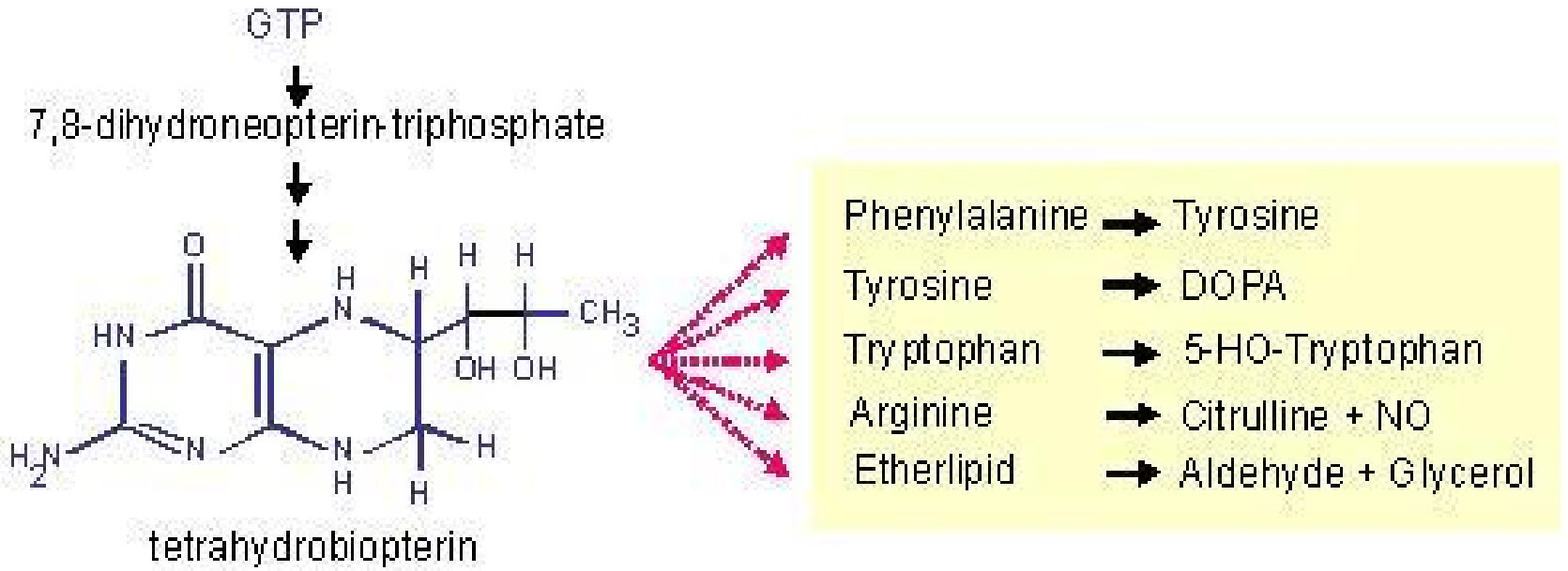

Figure 1: Biosynthesis and known cofactor roles of tetrahydrobiopterin

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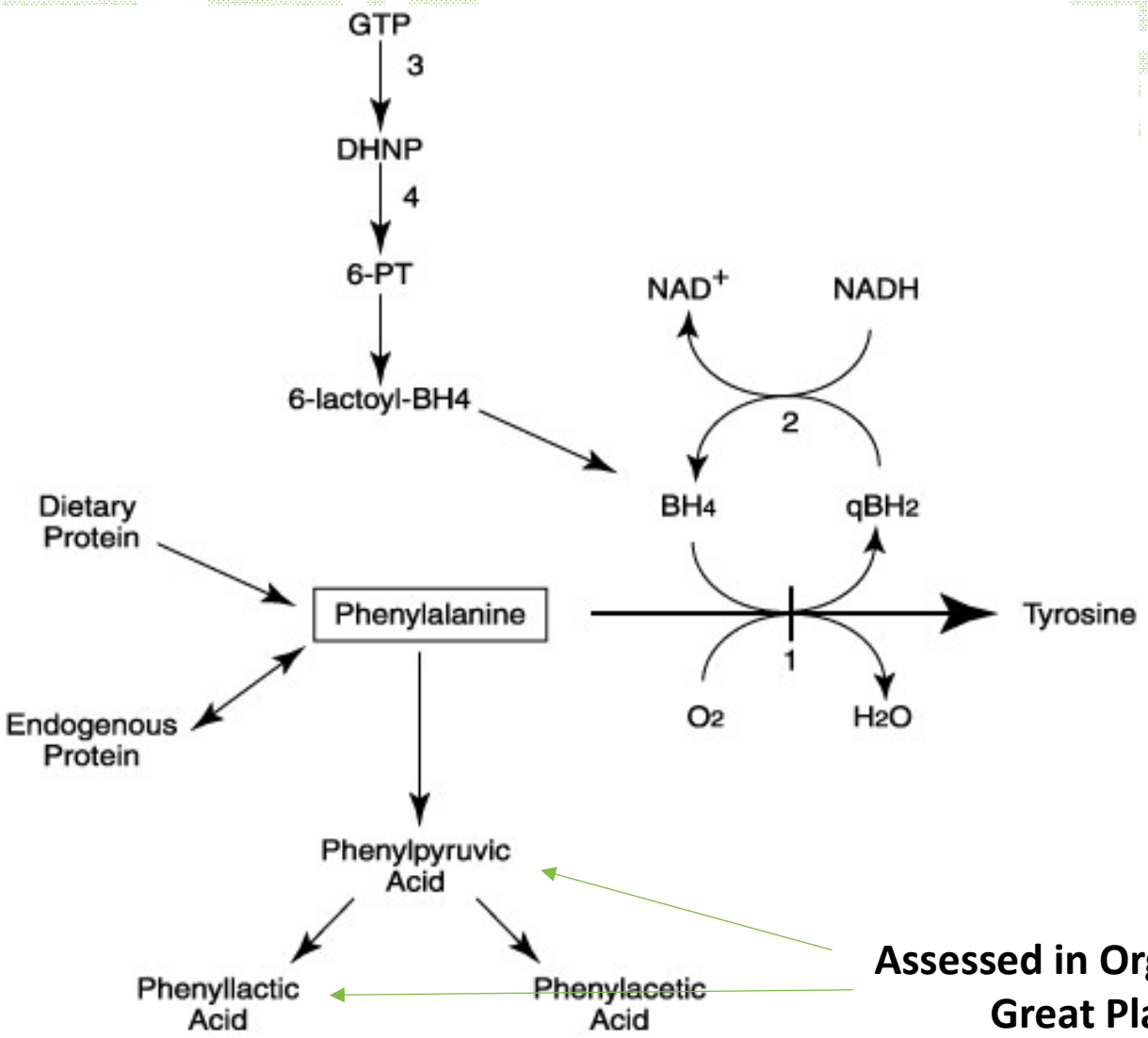

Tyrosine:

- Precursor to L-DOPA
- Precursor to Melanin
- Precursor to CoQ10
- Precursor to T3, T4
- Enzyme regulator

Assessed in Organic Acids Test from Great Plains Laboratory

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Clinical Suspicion For Poor BH4 Support of Tyrosine Production

- Low dopamine (HVA) and low norepinephrine (VMA)
- Low serotonin (5-HIAA)
- High ammonia
- Low thyroid
- Low energy, depressed or susceptible to depressed mood.
- Low energy
- Focus problems
- Pale skin *(tyrosine supports melanin production)*

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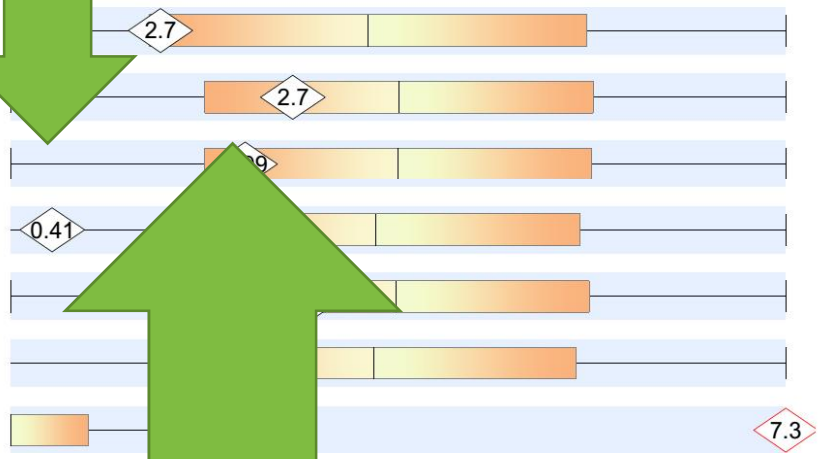
Commonly see low 5-HIAA

Neurotransmitter Metabolites

32	Homovanillic (HVA) *(dopamine)*		≤	14		2.7
33	Vanillylmandelic (VMA) *(norepinephrine, epinephrine)*	0.87	-	5.9		2.7
34	HVA / VMA Ratio	0.12	-	3.0		0.99
35	5-Hydroxyindoleacetic (5-HIAA) *(serotonin)*		≤	7.7		0.41
36	Quinolinic	0.63	-	6.7		3.0
37	Kynurenic		≤	4.1		1.3
38	Quinolinic / 5-HIAA Ratio	0.04	-	2.2	H	7.3



Lower the levels of HVA and VMA likely indicated problem in BH4 support of converting enzymes

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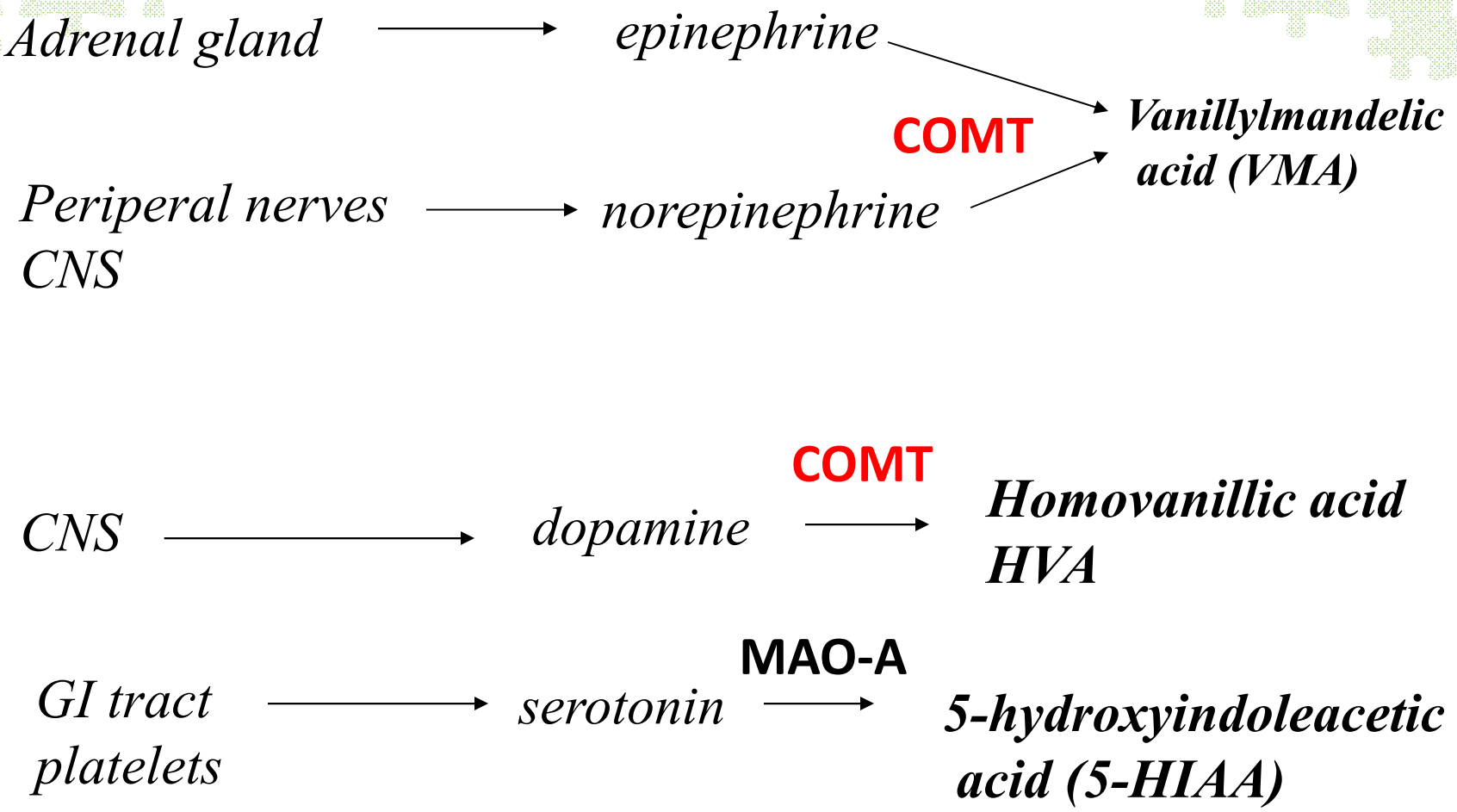
Neurotransmitter Metabolites



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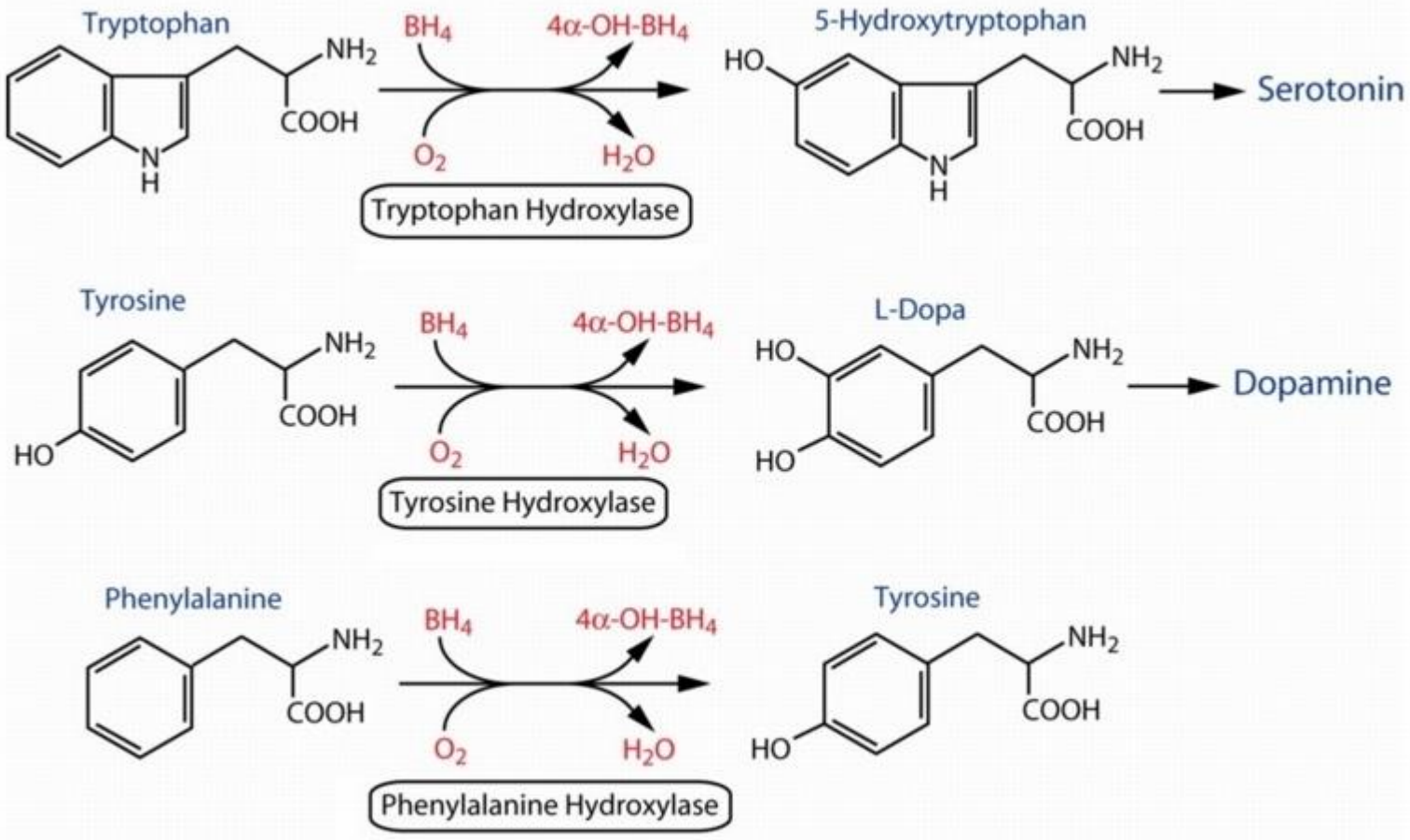
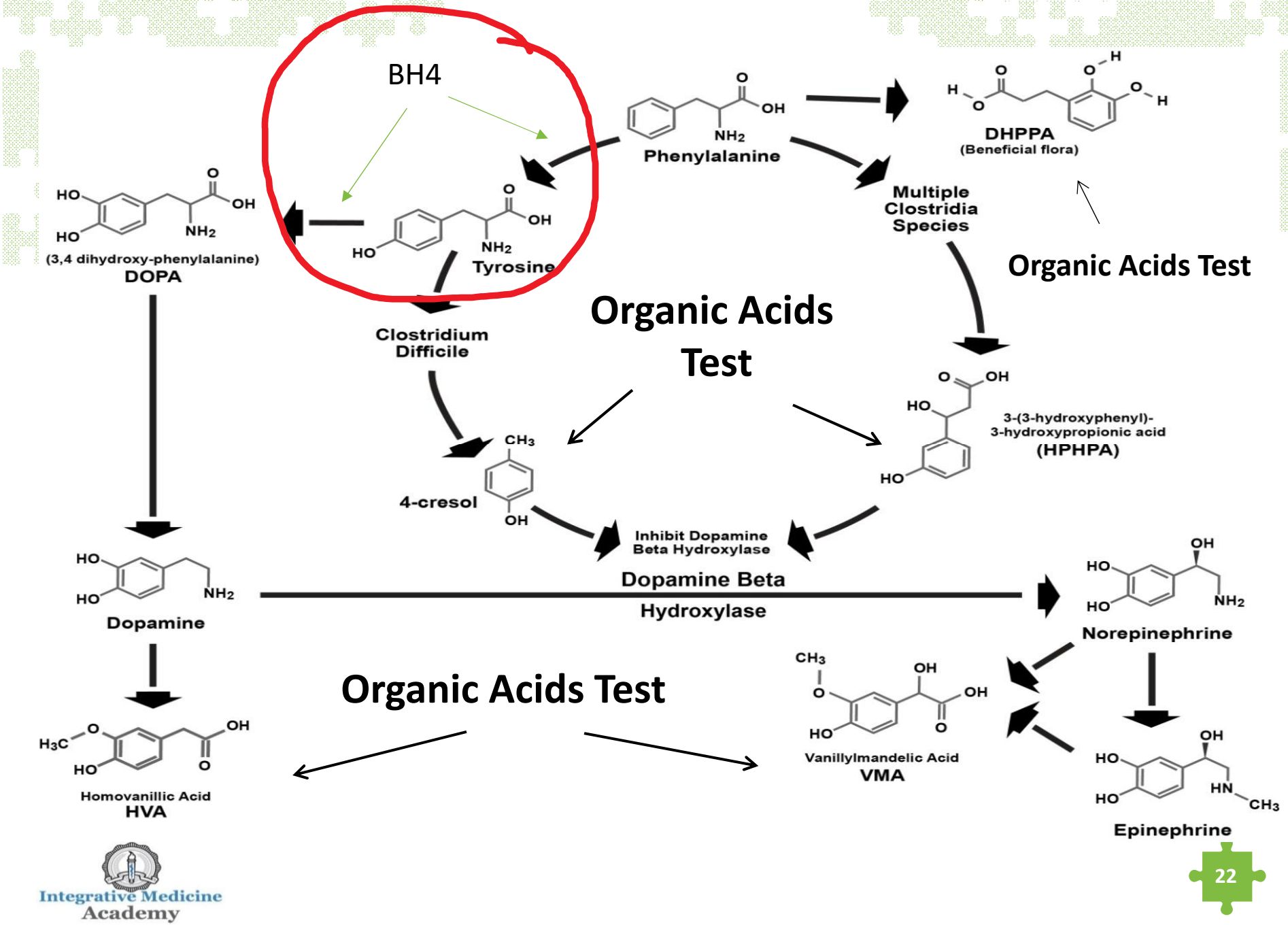
Reactions of Aromatic Amino Acid Hydroxylases with Biopterin



BH_4 = Tetrahydrobiopterin 4α-OH-BH_4 = Pterin 4 α-carbinolamine



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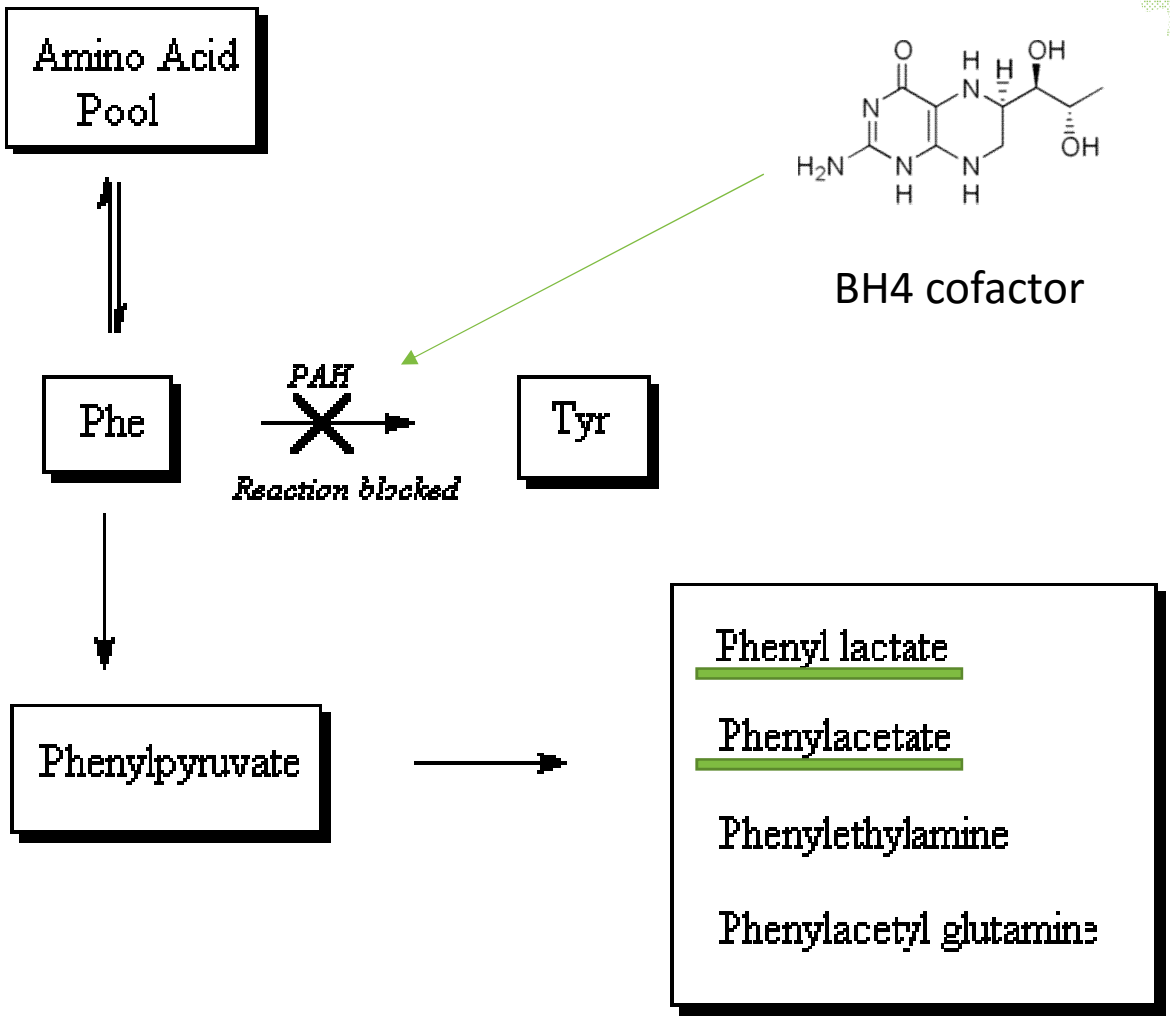
Abnormal Metabolism of Phenylalanine



BH4 cofactor

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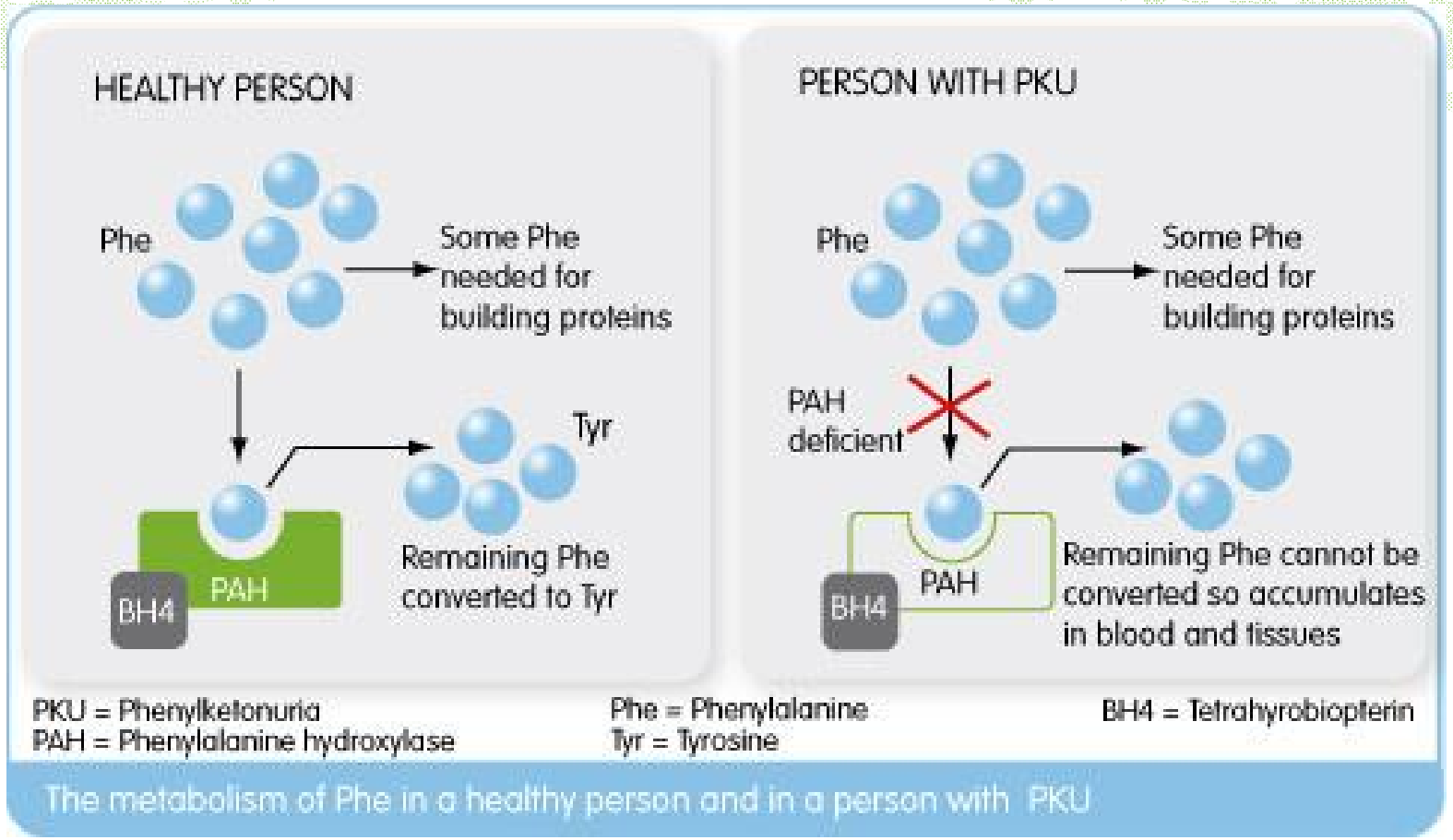
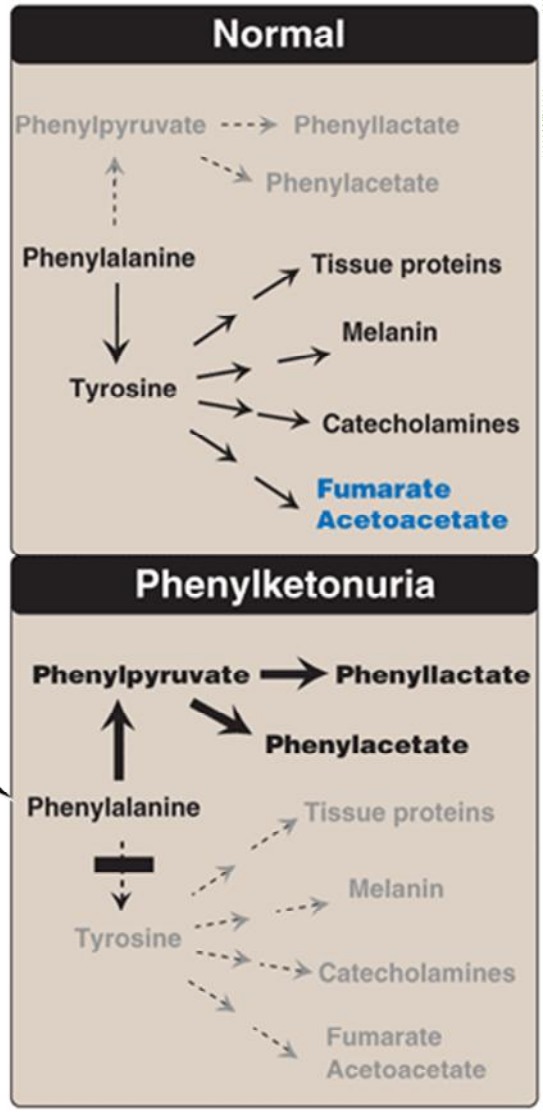


The metabolism of Phe in a healthy person and in a person with PKU

Symptoms

- Elevated phenylalanine, phenylpyruvate, phenyllactate and phenylacetate in blood and urine **(musty odor of urine).**
- **Neurological problems** (mental retardation, seizures, tremors, microcephaly etc) due to reduced production of catecholamines.
- **Hypopigmentation** (light skin, hair, blue eyes) due to reduced melatonin production.



Melanin, not Melatonin

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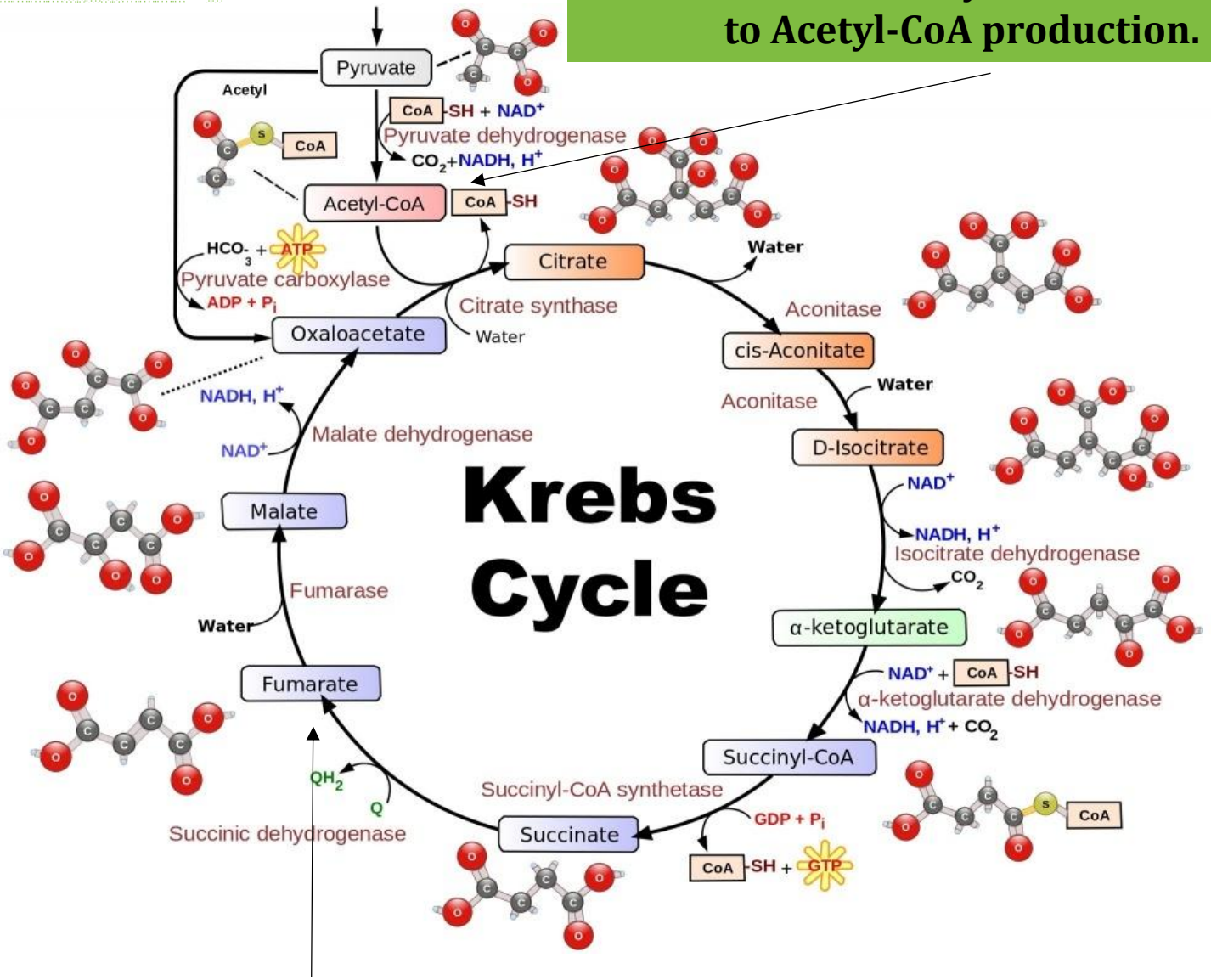
Acetoacetate from Tyrosine metabolism to Acetyl-CoA production.



Fumarate from Tyrosine metabolism

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Organic Acids Test – *Amino Acid Metabolites*

Phenyllactic Acid	A metabolite of phenylalanine. Elevated values indicate increased intake of dietary phenylalanine, or the heterozygous carrier status or homozygosity for the genetic disease phenylketonuria (PKU). Values observed in clinically diagnosed PKU typically exceed 200 mmol/mol creatinine.
Phenylpyruvic Acid	Moderate elevations may result from intake of phenylalanine, from genetic carrier status for PKU, or from a deficiency in production of biopterin, a cofactor required for phenylalanine metabolism. Very high values are associated with the genetic disease PKU.

Richard E. Frye, M.D., Ph.D. et.al. *Tetrahydrobiopterin as a novel therapeutic intervention for autism.* Neurotherapeutics. 2010 Jul; 7(3): 241–249.

- Tetrahydrobiopterin (BH_4) is an essential cofactor for several critical metabolic pathways that have been reported to be abnormal in autism spectrum disorder (ASD). In addition, the cerebrospinal fluid concentration of $BH_4$is reported to be depressed in children with ASD. Over the past 25 years, several clinical trials have suggested that treatment with BH_4improves ASD symptomatology in some individuals. Two ongoing clinical protocols may help further define the efficacy of BH_4 treatment in children with ASD. First, children with ASD who had low concentrations of cerebrospinal fluid or urine pterins were treated in an open-label manner with 20mg/kg/day of BH_4. The majority (63%) of children responded positively to treatment with minimal adverse effects. Second, a double-blind placebo-controlled study examining the efficacy of 20mg/kg/day of BH_4 treatment in children with ASD is currently underway. Safety studies from the commercially available forms of BH_4 document the low incidence of adverse effects, particularly serious adverse effects. Studies have also documented the ability of BH_4 to cross the blood brain barrier. Given the importance of BH_4 in neurodevelopmental metabolic pathways, the safety of BH_4 treatment, and the evidence for a therapeutic benefit of BH_4 treatment in children with ASD, we believe that BH_4 represents a novel therapy for ASD that may gain wider use after further clinical studies have established efficacy and treatment guidelines.

Dosage = 20mg/kg/day of BH4 *(this is a dose equivalent to the high end for PKU).*

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Kuvan (sapropterin dihydrochloride)

- Dihydrochloride salt of tetrahydrobiopterin (BH4)
- Acts as a Phenylalanine Hydroxylase activator
- Indicated for the use in Phenylketonuria to reduce Phenylalanine (Phe) levels.
- Comes in powder (100mg for oral solution) and tablets (100mg).
- 10mg/kg/day for one month. Recheck blood Phe after one week and then periodically for one month.
- No change in Phe, increase dose to 20/mg/kg/day. Use for additional 30 days and if no change discontinue.
- Maintenance dose – *5 to 20mg/kg/day*

BH4 (low dose option)

Tetrahydrobiopterin

- Low dose BH4 at 2.5mg capsules.
  - *1 to 4 capsules daily*
- Found from various online supplement stores.
- Can be prepared by various compounding pharmacies too, example:
  - *Hopewell Pharmacy (New Jersey – www.hopewellrx.com)*
  - *Lee-Silsby Pharmacy (www.leesilsby.com)*

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Tryptophan and the Connection to Quinolinic Acid

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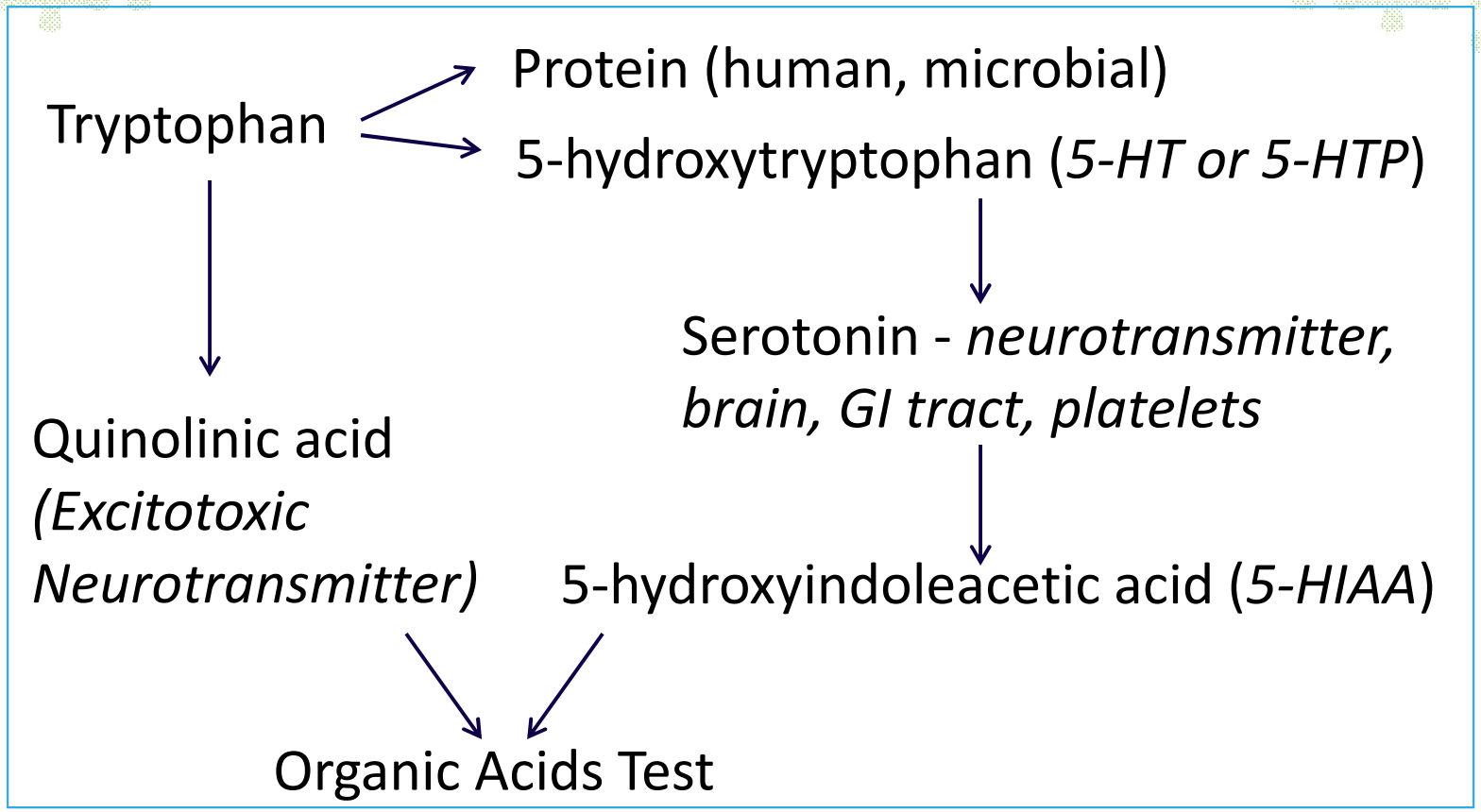
Tryptophan Need



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Where is Serotonin Produced?

- Approximately, 90% of serotonin is produced in the enterochromaffin cells of the intestine
- Nervous system
- Platelets

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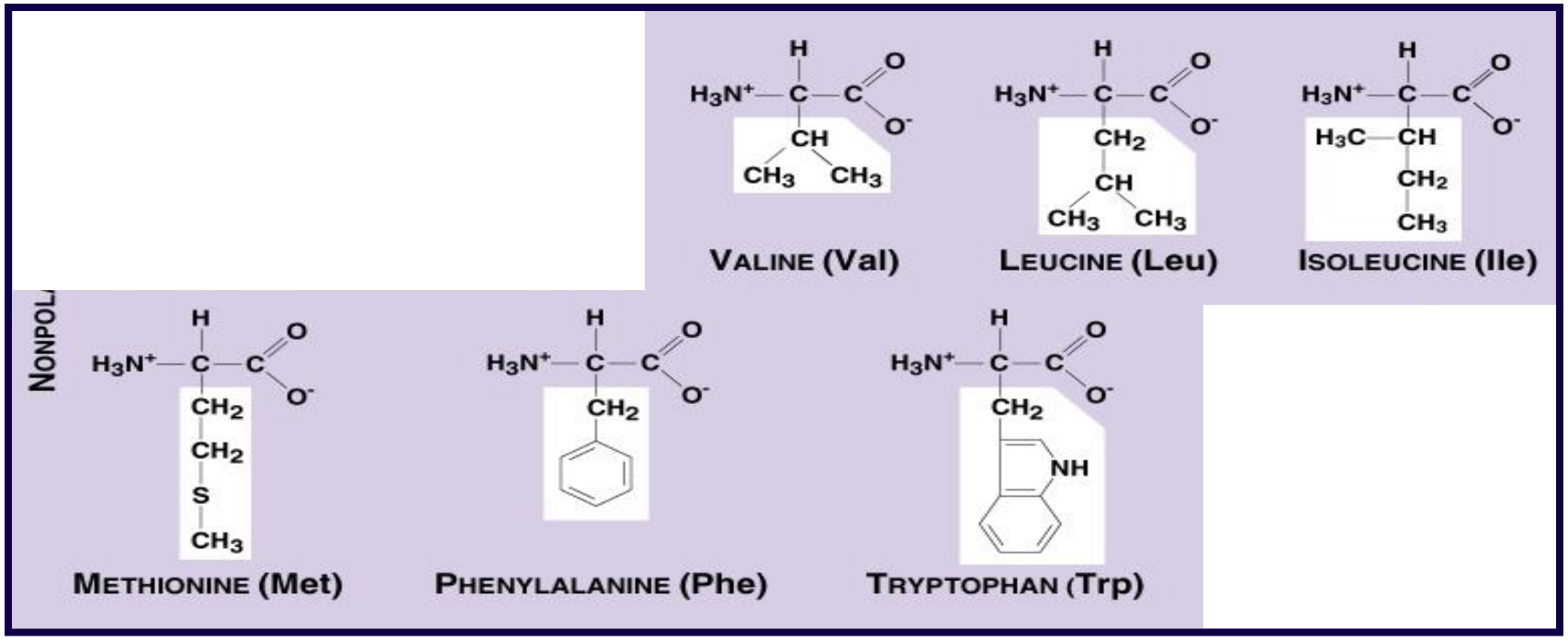
Large Neutral Amino Acids (LNAA) Compete with Tryptophan Entry into Brain



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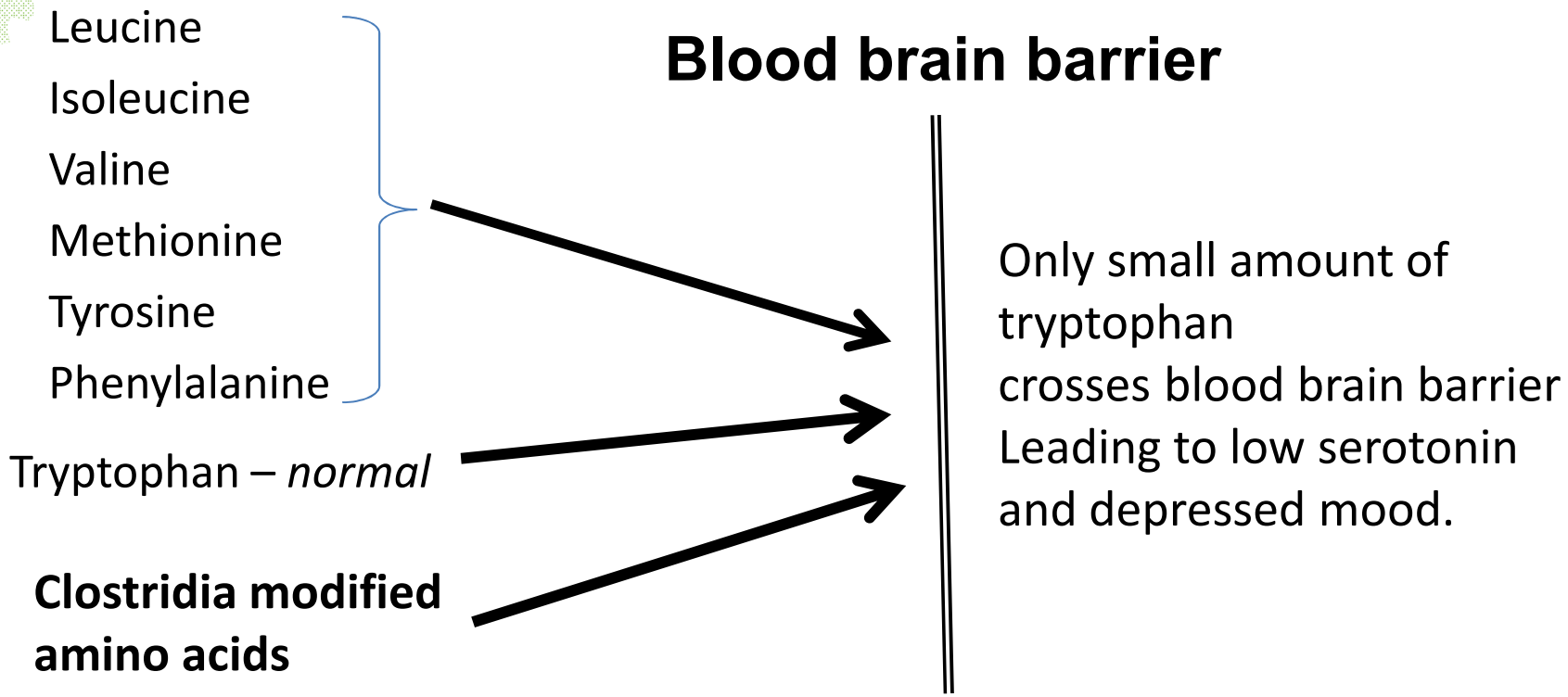
LNAA Amino Acids Reduce Tryptophan Brain Entry



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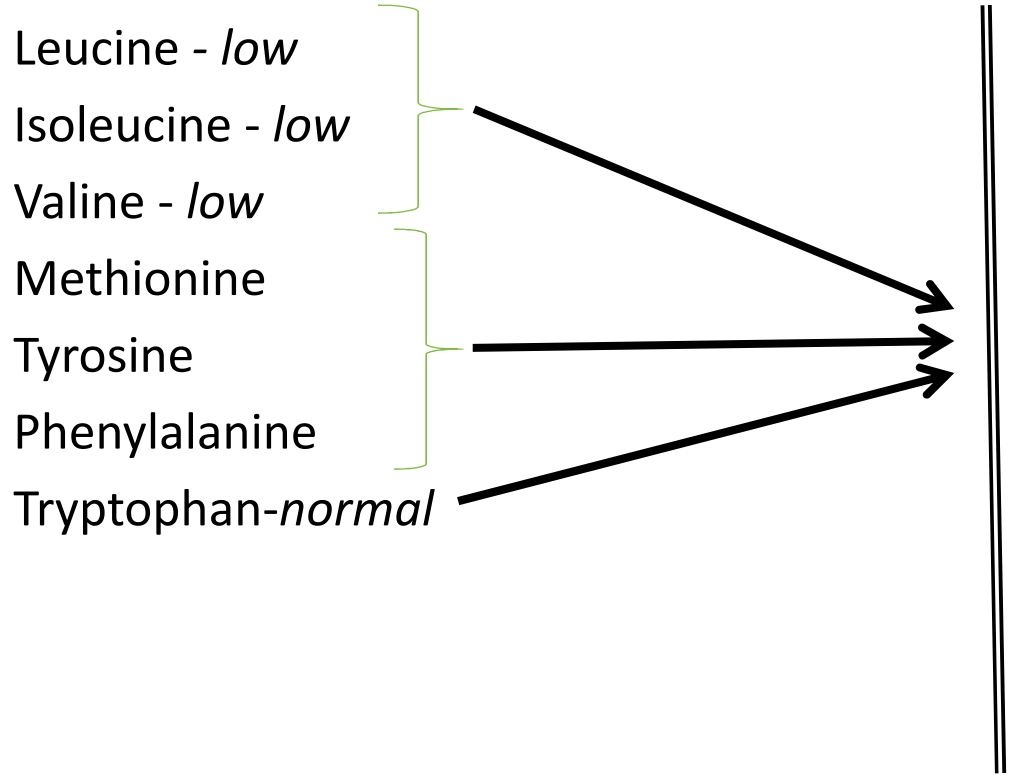
Carbohydrates Increase Brain Tryptophan Passage Across BBB

Blood Brain Barrier (BBB)



- High carbohydrate diet stimulates insulin leading to lowering of branched chain amino acids. With less competition, more tryptophan enters brain and increases serotonin leading to "feel-good" mood.
- **High protein diet reduces tryptophan entry into brain.**
- *Solution: use 5-HTP or tryptophan supplement for dieting.* ***5-HTP is better since it cannot produce quinolinic acid.***

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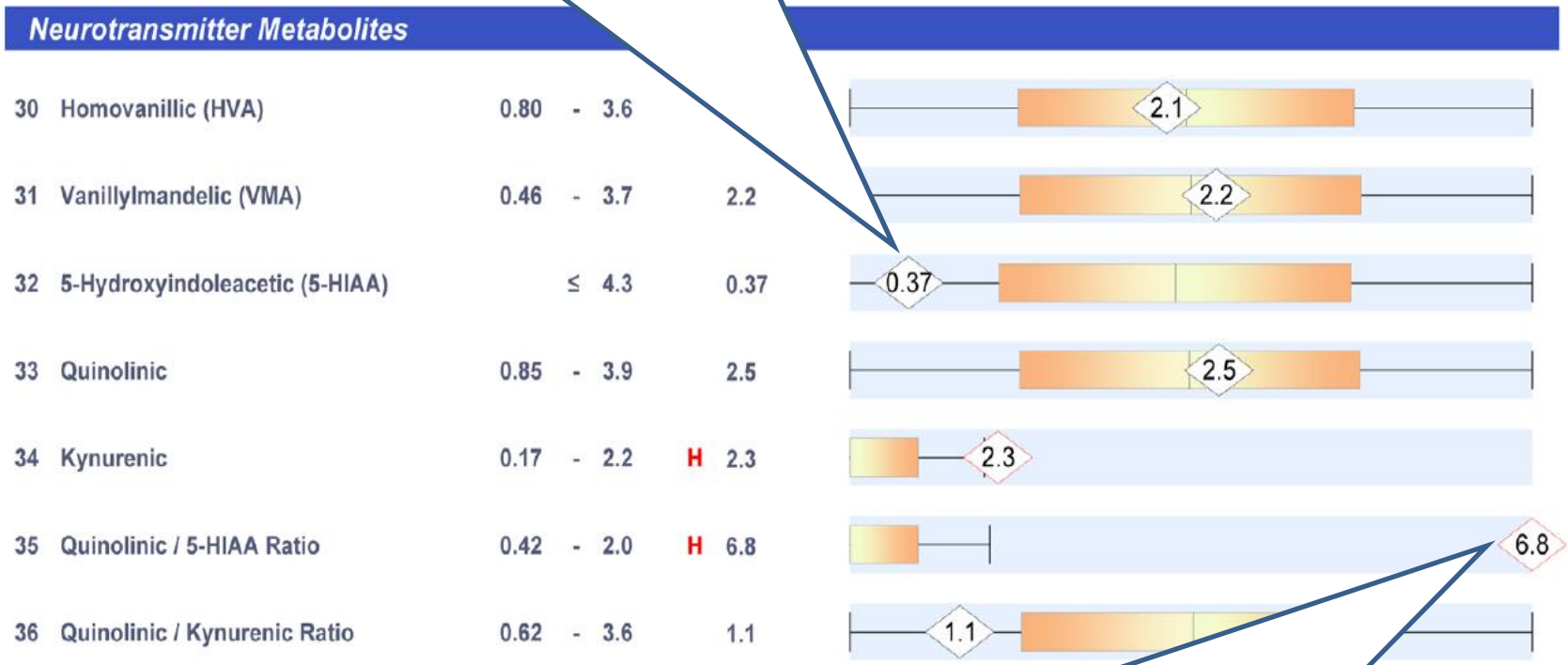
Takahashi S, et al. "Measurement of 5-hydroxyindole compounds during L-5-HTP treatment in depressed patients." *Folia Psychiatr Neurol Jpn.* 1976;30(4):461–73

- 150 mg 5-HTP orally for 7 days
- 7 of 14 patients responded to the treatment with mild or moderate amelioration of their depressive symptoms.
- Urinary excretion levels and plasma concentrations of 5-HIAA was measured during the drug treatment.
- Clinical response to L-5-HTP treatment appeared to have some correlation with the biochemical measures in the depressed patients, that is, non-responders exhibited significantly lower excretion of 5-HIAA in urine.

Low normal tryptophan metabolite for serotonin - *5-HIAA*



Neurotransmitter Metabolites				
30 Homovanillic (HVA)	0.80 - 3.6		2.1	
31 Vanillylmandelic (VMA)	0.46 - 3.7		2.2	
32 5-Hydroxyindoleacetic (5-HIAA)	≤ 4.3		0.37	
33 Quinolinic	0.85 - 3.9		2.5	
34 Kynurenic	0.17 - 2.2	H	2.3	
35 Quinolinic / 5-HIAA Ratio	0.42 - 2.0	H	6.8	
36 Quinolinic / Kynurenic Ratio	0.62 - 3.6		1.1	

Normal quinolinic acid, but very high ratio indicates increase conversion of tryptophan to quinolinic acid.

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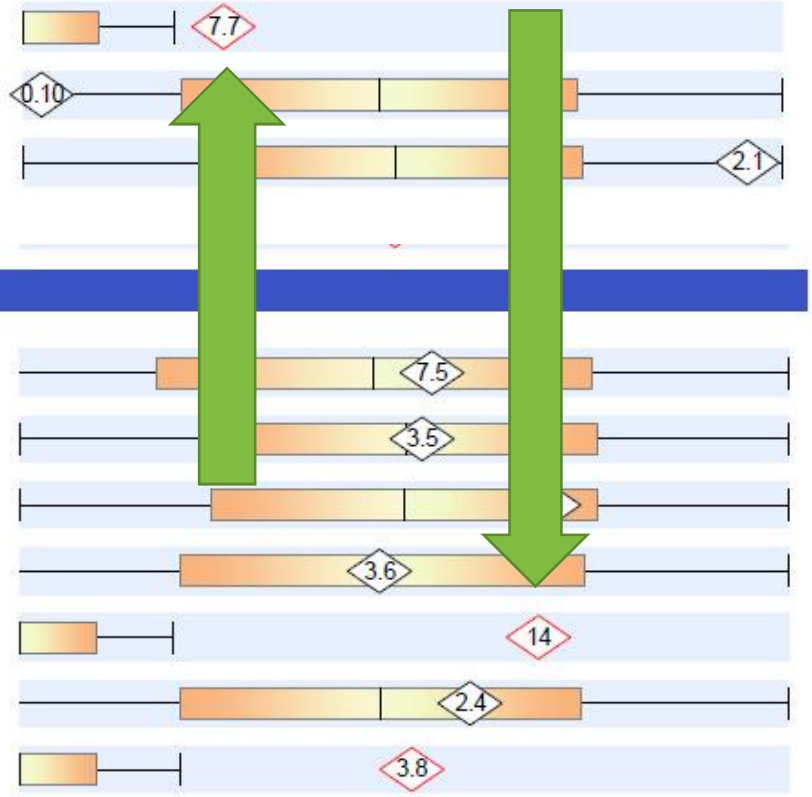
3 year old ASD child

Neurotransmitter Metabolites

32	Homovanillic (HVA) *(dopamine)*		≤ 14		12	12
33	Vanillylmandelic (VMA) *(norepinephrine, epinephrine)*	0.87	- 5.9		4.4	4.4
34	HVA / VMA Ratio	0.12	- 3.0		2.9	2.9
35	5-Hydroxyindoleacetic (5-HIAA) *(serotonin)*		≤ 7.7		3.7	3.7
36	Quinolinic	0.63	- 6.7	H	7.7	7.7
37	Kynurenic		≤ 4.1		0.10	0.10
38	Quinolinic / 5-HIAA Ratio	0.04	- 2.2		2.1	2.1

Neurotransmitter Metabolites

32	Homovanillic (HVA) *(dopamine)*		≤ 14		7.5	7.5
33	Vanillylmandelic (VMA) *(norepinephrine, epinephrine)*	0.87	- 5.9		3.5	3.5
34	HVA / VMA Ratio	0.12	- 3.0		2.1	
35	5-Hydroxyindoleacetic (5-HIAA) *(serotonin)*		≤ 7.7		3.6	3.6
36	Quinolinic	0.63	- 6.7	H	14	14
37	Kynurenic		≤ 4.1		2.4	2.4
38	Quinolinic / 5-HIAA Ratio	0.04	- 2.2	H	3.8	3.8



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5 year old ASD child

Various Adverse Effects of Elevated Quinolinic Acid

…and the link to Microglial Activation, Brain Inflammation, and NMDA receptor activation

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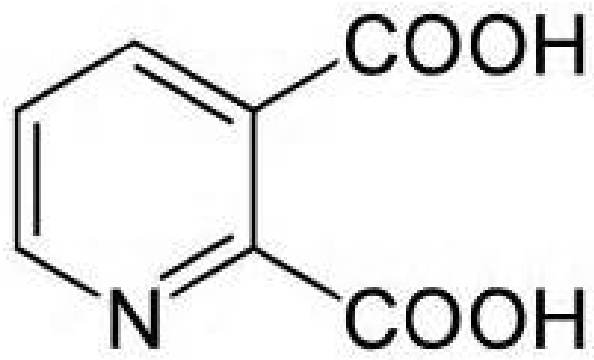
Quinolinic Acid

Quinolinic Acid

- Byproduct of tryptophan conversion after passing through kynurenine pathway.
- **NMDA receptor agonist**
- Potent neurotoxin
- Involved in neurodegeneration (i.e. Huntington's & Alzheimer's disease), psychiatric disorders (i.e. depression, mood disorders, schizophrenia), body pain, suicide…



Dicarboxylic acid

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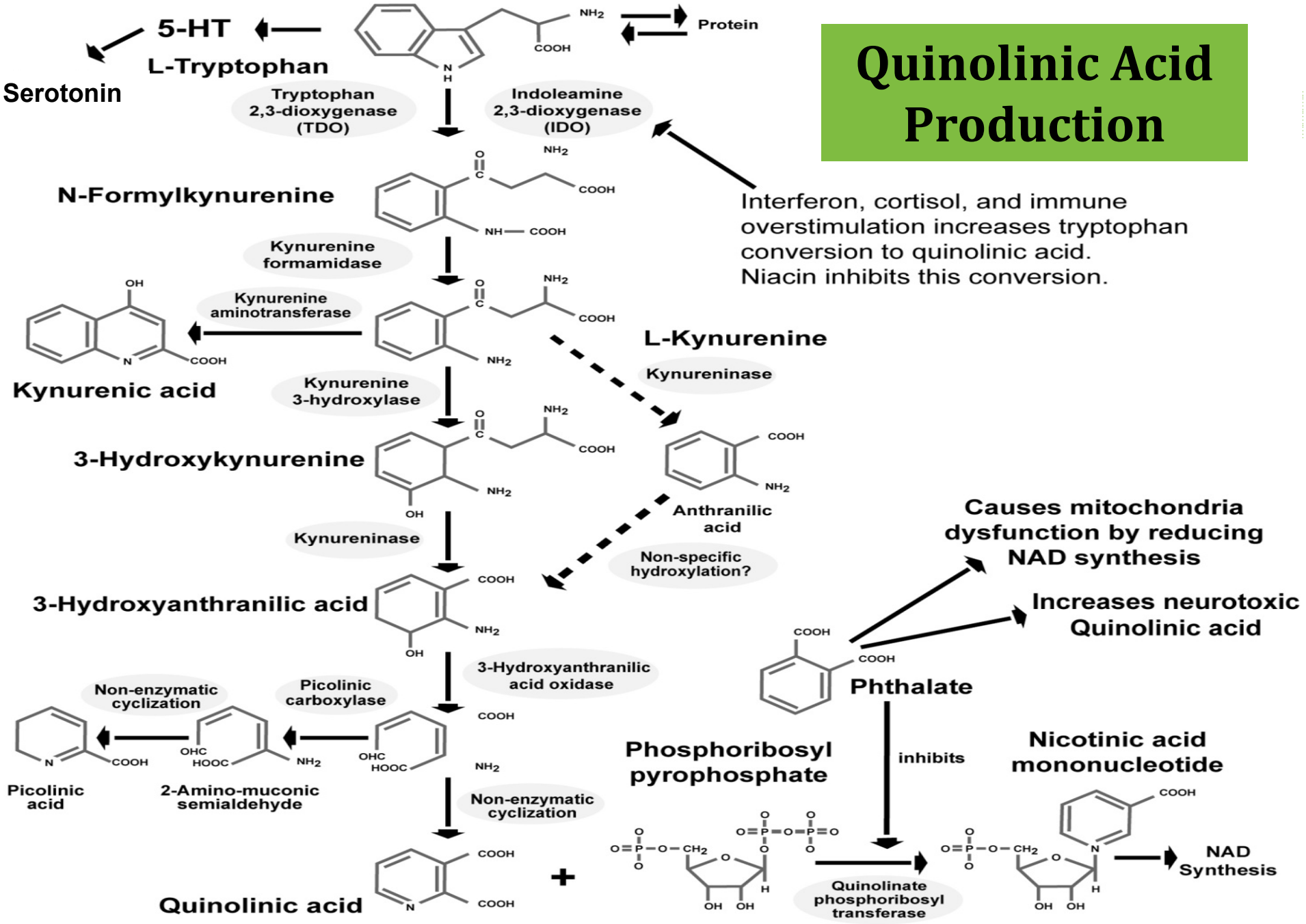
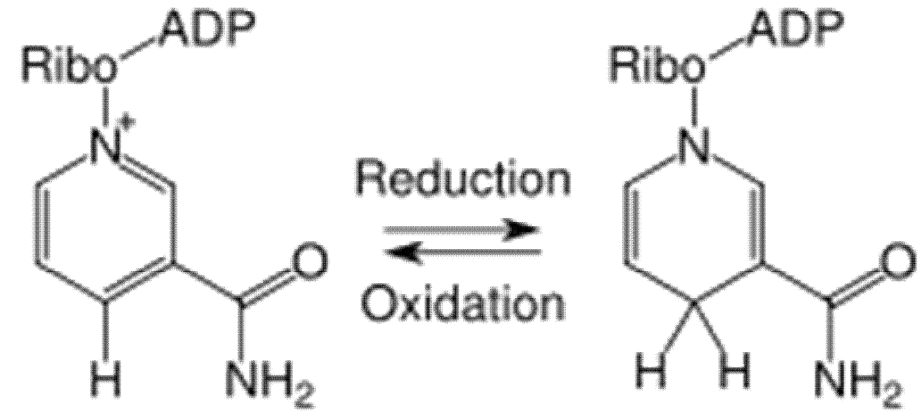
Quinolinic Acid Production



Nicotinamide adenine dinucleotide



$NAD^+ + H^+ + 2e^- \longrightarrow NADH$

Oxidized

Reduced

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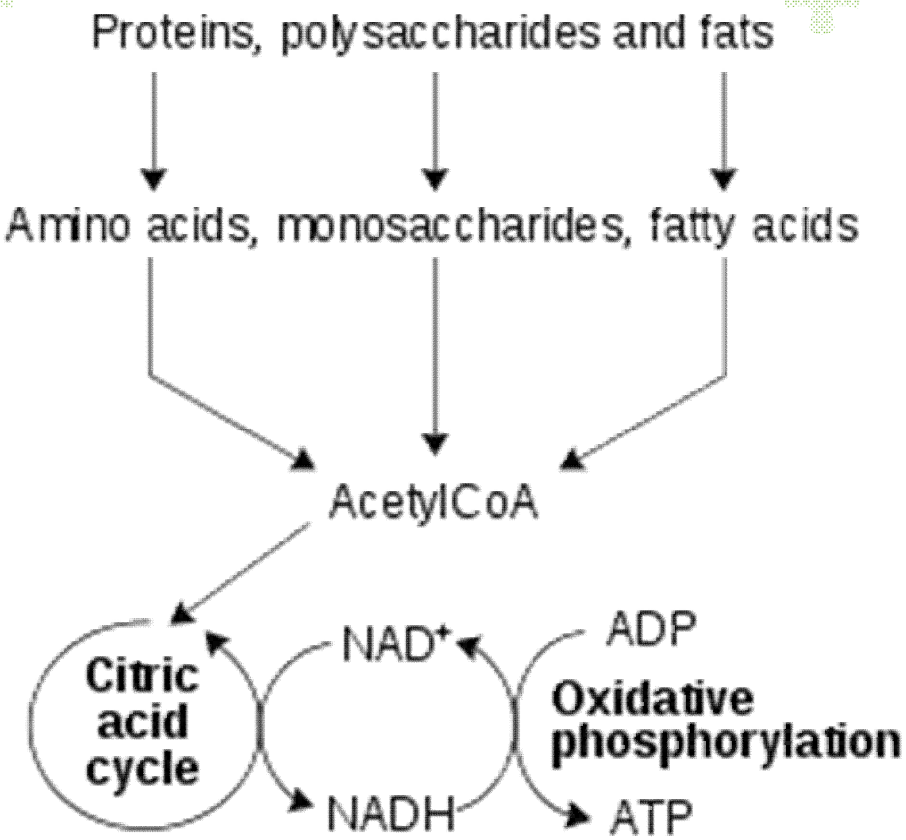

Redox Reaction

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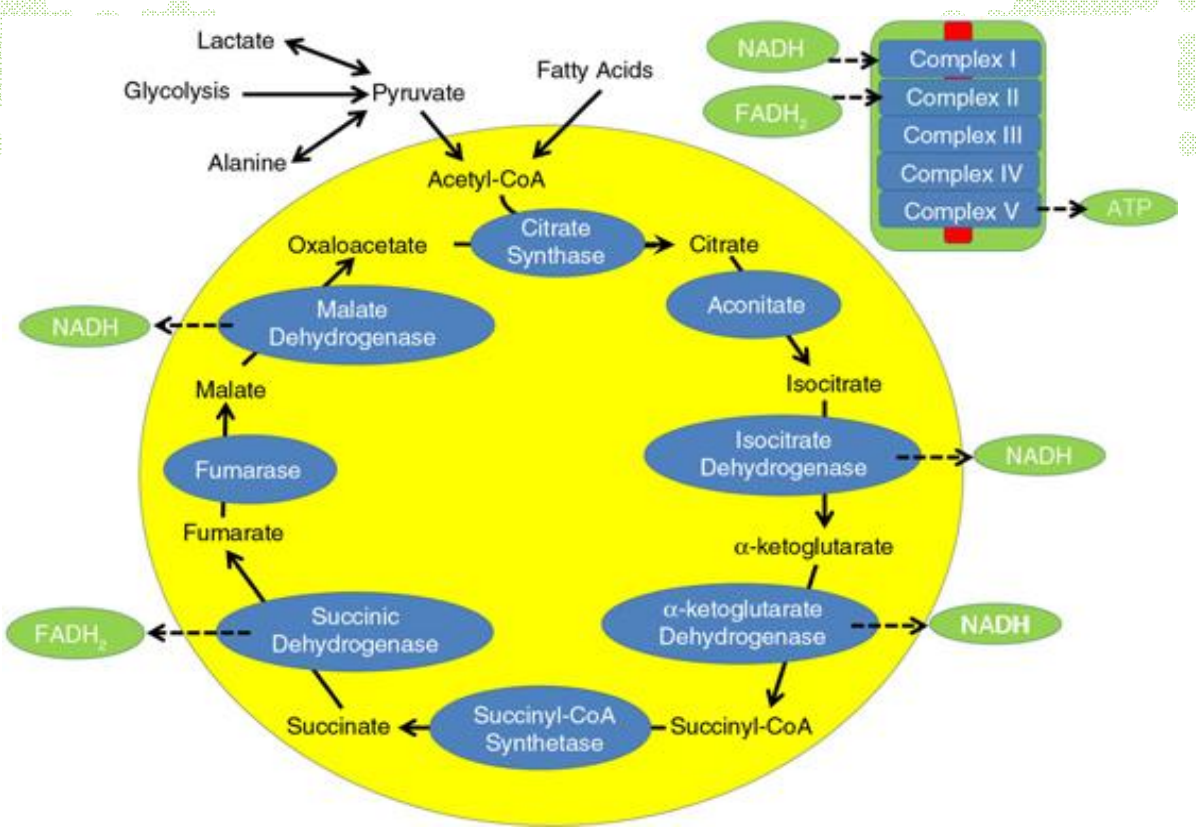
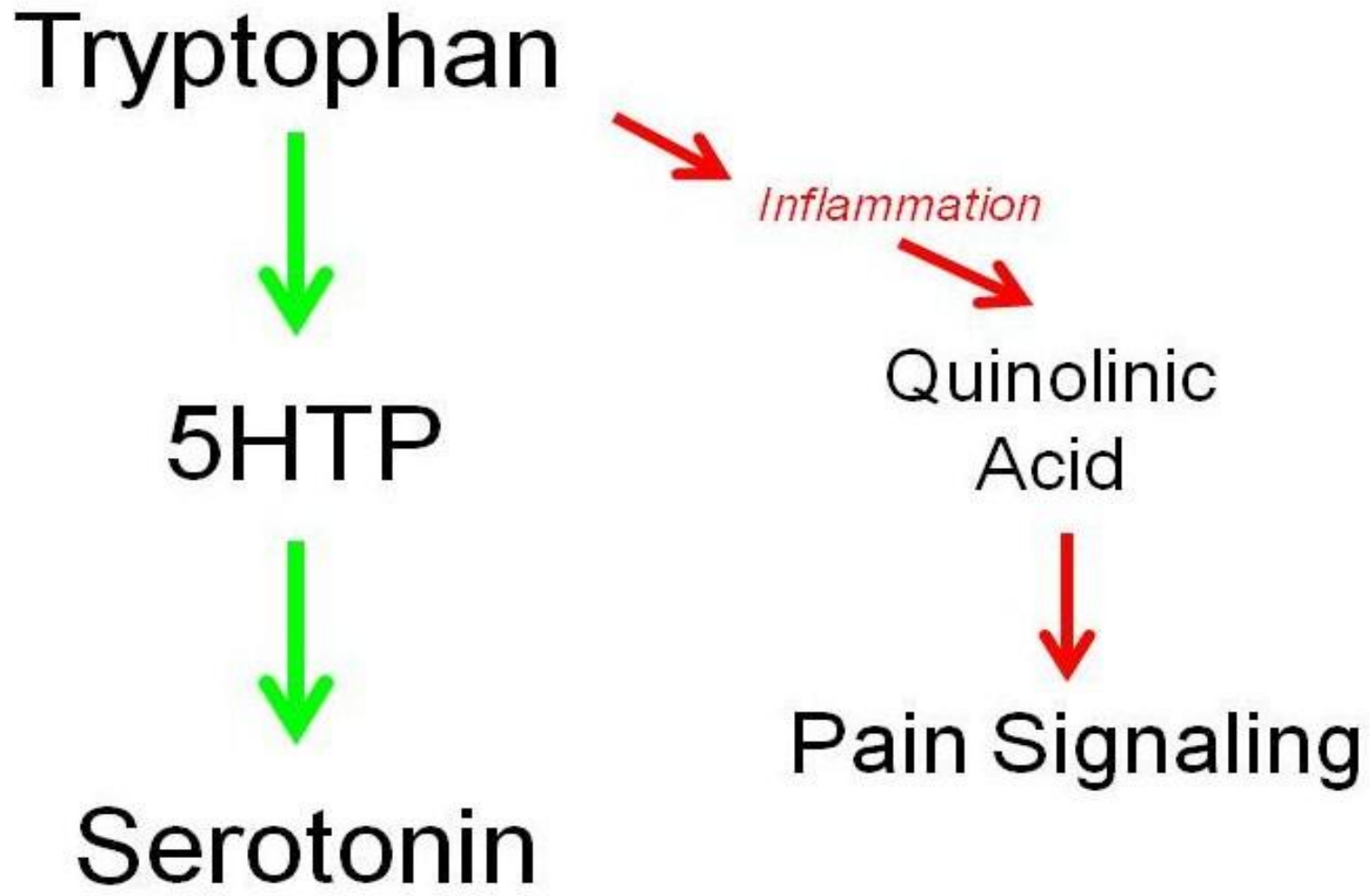


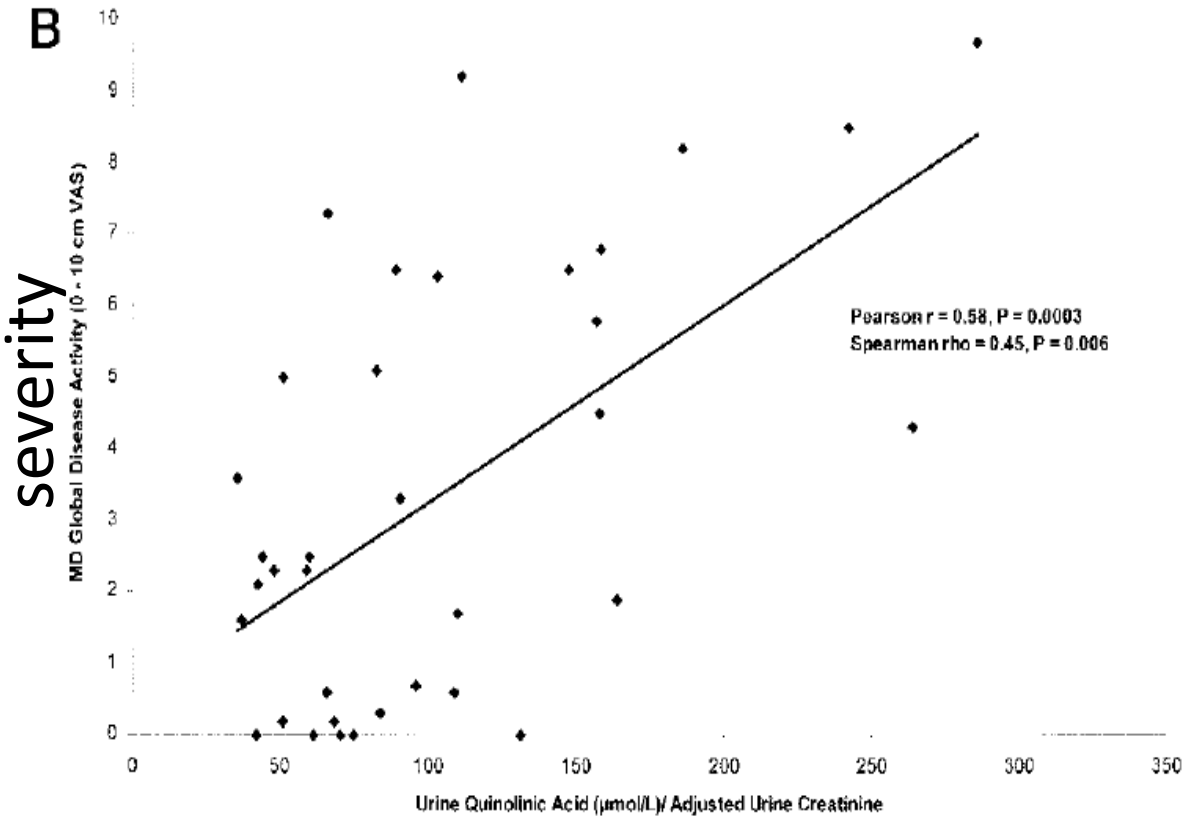
Fig. 1. The tricarboxylic acid cycle during typical metabolism. Carbohydrates and fatty acids enter the cycle as acetyl-CoA and through a series of enzymatic steps produce energy utilizing two electron carriers, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$). NADH and $FADH_2$ are metabolized by complex I and complex II, respectively, of the electron transport chain (ETC). Complex V of the ETC produces adenosine triphosphate (ATP), the energy carrier of the cell.

From: Gastrointestinal dysfunction in autism spectrum disorders: the role of the mitochondria and the enteric microbiome (2015)


Tryptophan
Inflammation
5HTP
Quinolinic Acid
Pain Signaling
Serotonin

Clinical Chemistry 48, No. 10, 2002

Arthritis global disease severity



Urine Quinolinic acid/creatinine

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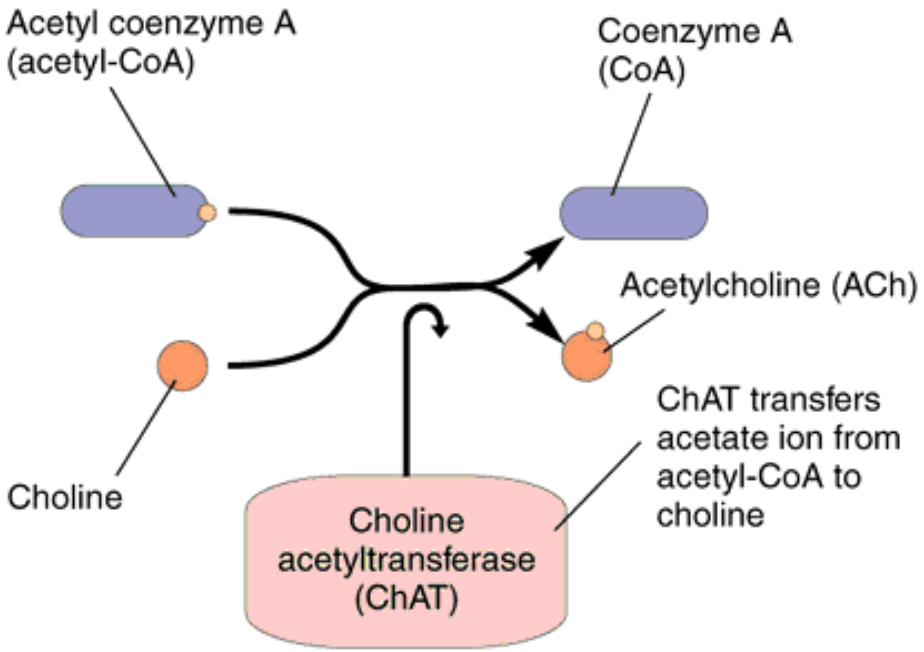
► **Biosynthesis of Acetylcholine**



Inhibited by Quinolinic Acid

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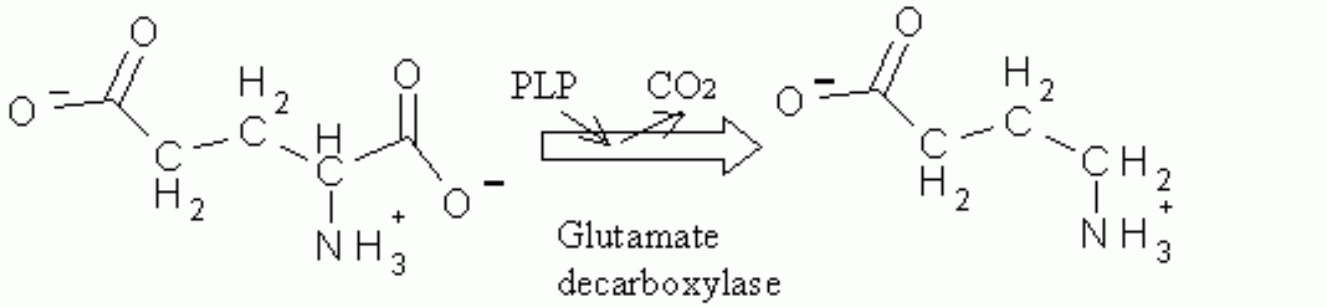

Glutamate **gamma-aminobutyric acid**

Figure 2 Synthesis of GABA

Inhibited by Quinolinic Acid

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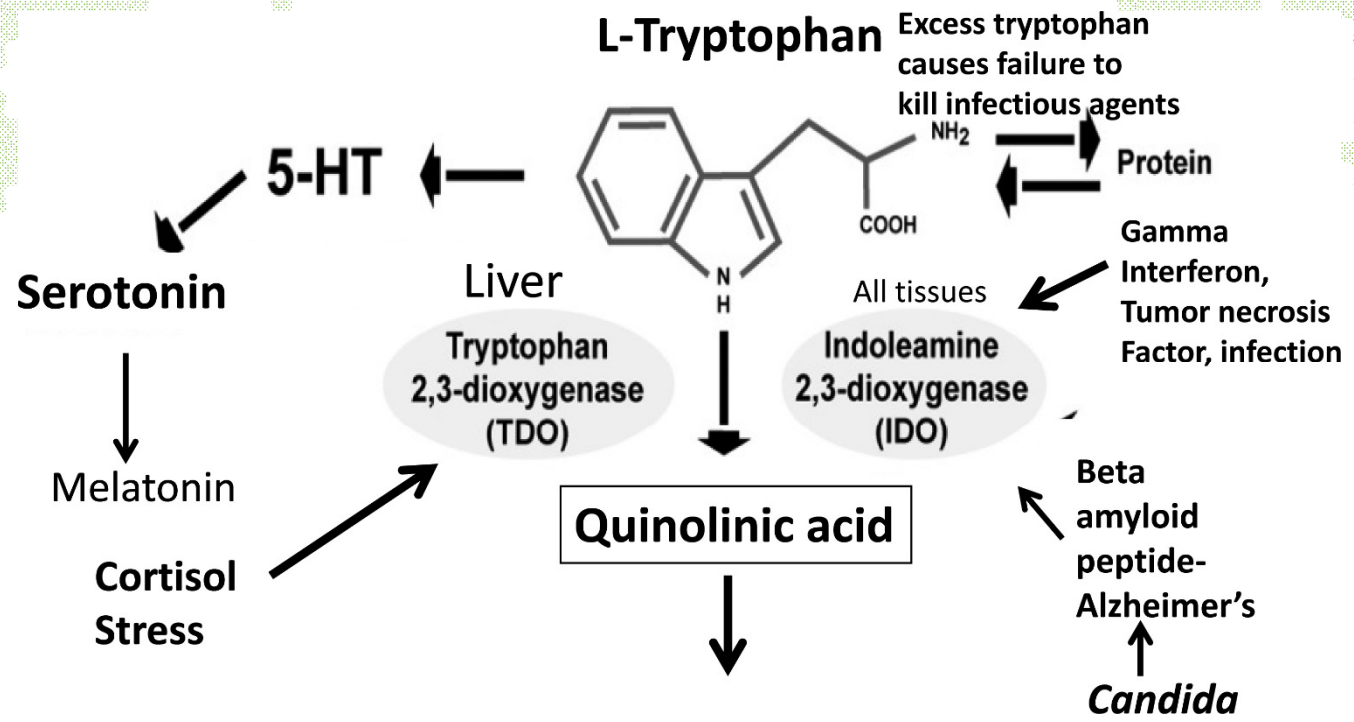
Neurology. 1992 Jan;42(1):43-50.
Neuroactive kynurenines in Lyme borreliosis.
Halperin JJ, Heyes MP.

Although neurologic dysfunction occurs frequently in patients with Lyme borreliosis, it is rarely possible to demonstrate the causative organism within the neuraxis. This discordance could arise if neurologic symptoms were actually due to soluble neuromodulators produced in response to infection. Since immune stimulation is associated with the production of quinolinic acid (QUIN), an excitotoxin and N-methyl-D-aspartate (NMDA) agonist, we measured levels of CSF and serum QUIN, and lymphokines. Samples were obtained from 16 patients with CNS Borrelia burgdorferi infection, eight patients with Lyme encephalopathy (confusion without intra-CNS inflammation), and 45 controls. CSF QUIN was substantially elevated in patients with CNS Lyme and correlated strongly with CSF leukocytosis. In patients with encephalopathy, serum QUIN was elevated with corresponding increments in CSF QUIN. Lymphokine concentrations were not consistently elevated. We conclude that CSF QUIN is significantly elevated in B burgdorferi infection--dramatically in patients with CNS inflammation, less in encephalopathy. The presence of this known agonist of NMDA synaptic function--a receptor involved in learning, memory, and synaptic plasticity--may contribute to the neurologic and cognitive deficits seen in many Lyme disease patients

We conclude that CSF QUIN is significantly elevated in B. burgdorferi infection - *dramatically in patients with CNS inflammation, less in encephalopathy.*



Kills cells containing bacteria, viruses, parasites. May also damage infectious organisms themselves.

IDO causes drastic reduction in tryptophan for protein synthesis. Tryptophan needed by infected cells and infectious organisms.

Generally, tryptophan naturally kept at low levels to protect against microbes.

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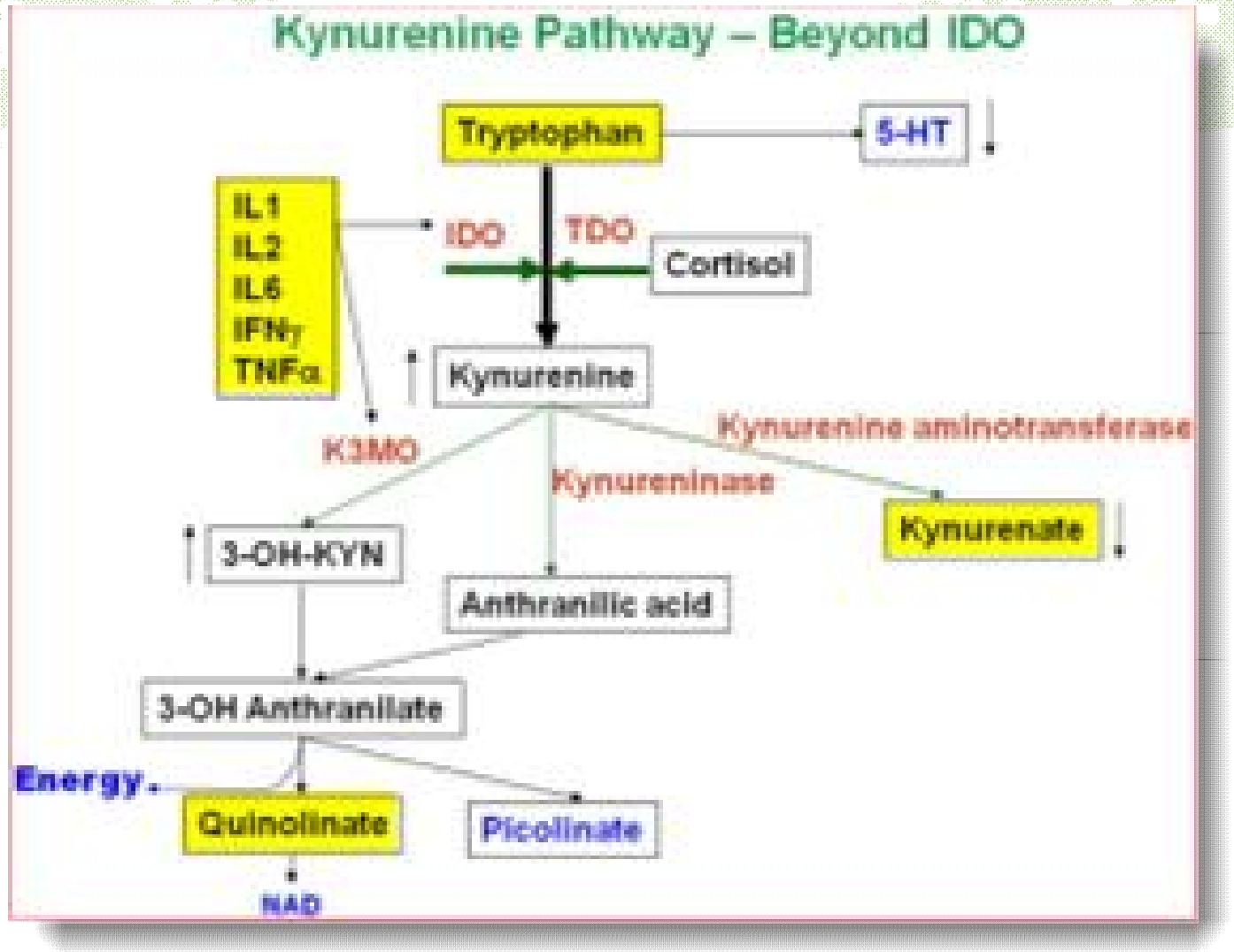

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IDO activating Cytokines

- Interleukin 1 (most common are IL-1$^{\alpha}$ and IL-1β):
  - *Produced by macrophages, monocytes and dendritic cells.*
  - *Fever production, hyperalgesia and vasodilation*
  - *Pro-inflammatory*
- Interleukin 2:
  - *Control over the immune system primarily by regulating T-cells.*
  - *Plays a role in "ramping up" and "ramping down" the immune response.*

IDO Activating Cytokines

- Interleukin 6:
  - *Pro-inflammatory cytokine in response to infection*
  - *Has Hypothalamus-Pituitary-Adrenal (HPA) axis influence by stimulating ACTH production.*
- Interferon-γ:
  - *Produced primarily from natural killer cells in response to viral infections.*
- Tumor Necrosis Factor-α:
  - *Produced by macrophages, NK cells, eosinophils, etc.*
  - *Regulated by IL-1 and IL-6*
  - *Pro-inflammatory*
  - *Implicated in inflammatory bowel disease, depression, Alzheimer's disease, and cancer.*

Ashwood P et.al. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun. 2011 Jan;25(1):40-5

Autism spectrum disorders (ASD) are characterized by impairment in social interactions, communication deficits, and restricted repetitive interests and behaviors. A potential role for immune dysfunction has been suggested in ASD. To test this hypothesis, we investigated evidence of differential cytokine release in plasma samples obtained from 2 to 5 year-old children with ASD compared with age-matched typically developing (TD) children and children with developmental disabilities other than autism (DD). Participants were recruited as part of the population based case-control CHARGE (Childhood Autism Risks from Genetics and Environment) study and included: 97 participants with a confirmed diagnosis of ASD using standard assessments (DSM IV criteria and ADOS, ADI-R), 87 confirmed TD controls, and 39 confirmed DD controls. Plasma was isolated and cytokine production was assessed by multiplex Luminex™ analysis. Observations indicate significant increases in plasma levels of a number of cytokines, including IL-1β, IL-6, IL-8 and IL-12p40 in the ASD group compared with TD controls ($p<0.04$). Moreover, when the ASD group was separated based on the onset of symptoms, it was noted that the increased cytokine levels were predominantly in children who had a regressive form of ASD. In addition, increasing cytokine levels were associated with more impaired communication and aberrant behaviors. In conclusion, using larger number of participants than previous studies, we report significantly shifted cytokine profiles in ASD. These findings suggest that ongoing inflammatory responses may be linked to disturbances in behavior and require confirmation in larger replication studies. The characterization of immunological parameters in ASD has important implications for diagnosis, and should be considered when designing therapeutic strategies to treat core symptoms and behavioral impairments of ASD.

"Observations indicate significant increases in plasma levels of a number of cytokines, including IL-1β, IL-6, IL-8 and IL-12p40 in the ASD group compared with TD controls ($p<0.04$). Moreover, when the ASD group was separated based on the onset of symptoms, <u>it was noted that the increased cytokine levels were predominantly in children who had a regressive form of ASD.</u>"

Integrative Medicine Academy

Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children. Pediatric Neurology. 07/2007; 36(6):361-5

Recent reports implicating elevated cytokines in the central nervous system in a small number of patients studied with autism have reported clinical regression. These studies have not focused on tumor necrosis factor-alpha as a possible marker for inflammatory damage. A series of 10 children with autism had clinical evaluation of their serum and spinal fluid for inflammatory changes and possible metabolic disease as part of their neurological evaluation. Elevation of cerebrospinal fluid levels of tumor necrosis factor-alpha was significantly higher (mean = 104.10 pg/mL) than concurrent serum levels (mean = 2.78 pg/mL) in all of the patients studied. The ratio of the cerebrospinal fluid levels to serum levels averaged 53.7:1. This ratio is significantly higher than the elevations reported for other pathological states for which cerebrospinal fluid and serum tumor necrosis factor-alpha levels have been simultaneously measured. This observation may offer a unique insight into central nervous system inflammatory mechanisms that may contribute to the onset of autism and may serve as a potential clinical marker. More controlled study of this potentially important observation may prove valuable.

Elevation of cerebrospinal fluid levels of tumor necrosis factor-alpha was significantly higher (mean = 104.10 pg/mL) than concurrent serum levels (mean = 2.78 pg/mL) in all of the patients studied.

Neurological Inflammation

Neuroglial activation and neuroinflammation in the brain of patients with autism.

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA.

Department of Neurology, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Baltimore, MD 21287, USA.

Autism is a neurodevelopmental disorder characterized by impaired communication and social interaction and may be accompanied by mental retardation and epilepsy. Its cause remains unknown, despite evidence that genetic, environmental, and immunological factors may play a role in its pathogenesis. To investigate whether immune-mediated mechanisms are involved in the pathogenesis of autism, we used immunocytochemistry, cytokine protein arrays, and enzyme-linked immunosorbent assays to study brain tissues and cerebrospinal fluid (CSF) from autistic patients and determined the magnitude of neuroglial and inflammatory reactions and their cytokine expression profiles. Brain tissues from cerebellum, midfrontal, and cingulate gyrus obtained at autopsy from 11 patients with autism **(age 5 to 44 years old)** were used for morphological studies. Fresh-frozen tissues available from seven patients and CSF from six living autistic patients were used for cytokine protein profiling. We demonstrate an **active neuroinflammatory process in the cerebral cortex, white matter, and notably in cerebellum of autistic patients**. Immunocytochemical studies showed marked activation of microglia and astroglia, and cytokine profiling indicated that macrophage chemoattractant protein (MCP)-1 and tumor growth factor-beta1, derived from neuroglia, were the most prevalent cytokines in brain tissues. CSF showed a unique proinflammatory profile of cytokines, including a marked increase in MCP-1.

Our findings indicate that innate neuro-immune reactions play a pathogenic role in an undefined proportion of autistic patients, suggesting that future therapies might involve modifying neuroglial responses in the brain.

Ann Neurol. 2005 Feb;57(2):304

Vargas DL., et.al Study (*John Hopkins*)

- Examined tissue from 3 different regions of the brain in 11 deceased individuals with autism – ages 5 to 44 (who died of accidents or injuries)
- Measured cytokine & chemokine from cerebrospinal fluid in 6 living individuals with autism – ages 5 to 12

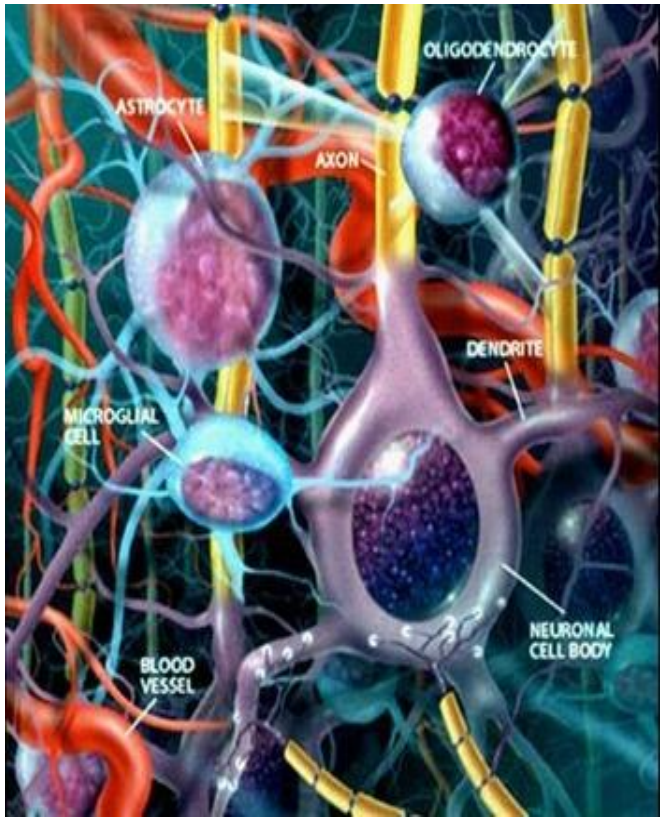
Findings:

- Active neuroinflammatory process in the cerebral cortex, white matter, and notably in cerebellum of autistic patients.
- Marked activation of microglia and astroglia.
- Macrophage chemoattractant protein 1 (MCP-1) and tumor growth factor-beta1, derived from neuroglia, were the most prevalent cytokines in brain tissues.
- CSF showed a unique pro-inflammatory profile of cytokines, including a marked increase in MCP-1.

"Our findings indicate that innate neuroimmune reactions play a pathogenic role in an undefined proportion of autistic patients, suggesting that future therapies might involve modifying neuroglial responses in the brain."

Astroglia & Microglia

- **Astroglia**
  - Physical support of brain
  - Support blood brain barrier
  - Brain nutrient support
  - Brain repair and scarring
- **Microglia**
  - **Main immune defense in the brain**.
  - 20% of glial cells in the brain
  - Microglia cells are constantly on the move looking for invading pathogens.
  - They initiate the inflammatory response
  - Present foreign virus, bacteria, etc. to immune system.
  - **Can become chronically activated and never turn off.**



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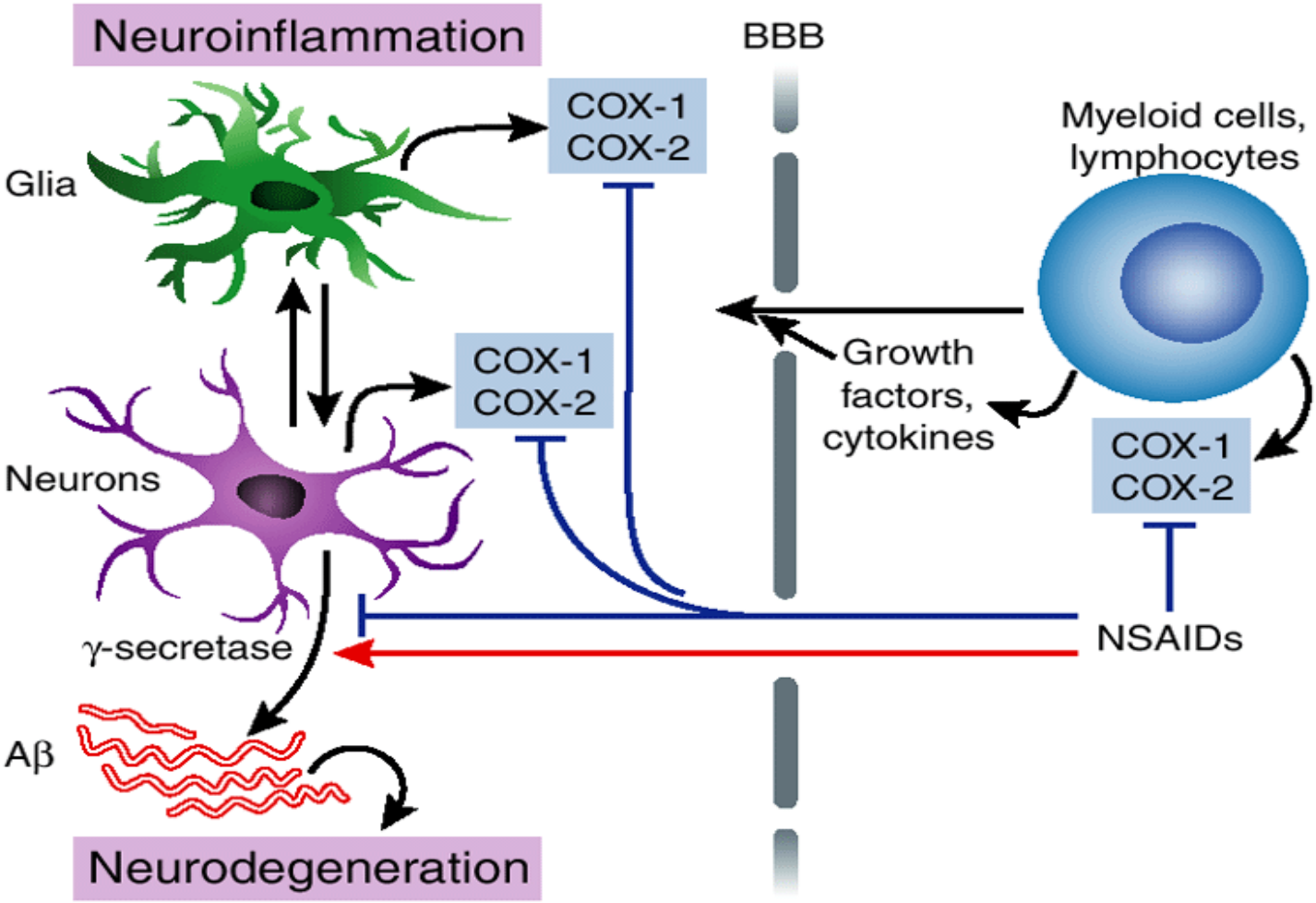
Neuroinflammation



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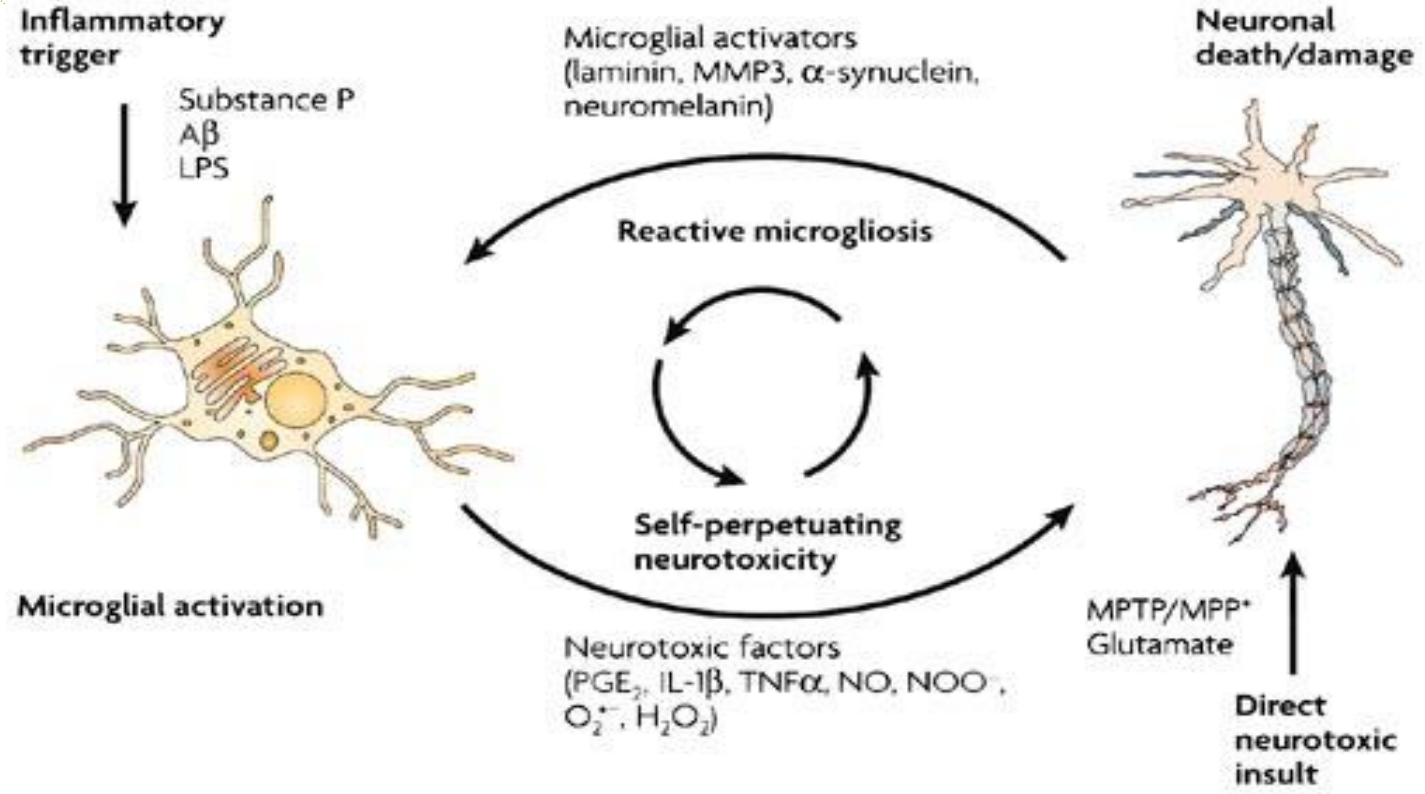
Microglial Activation



Direct and Indirect activation of microglia leads to cell damage.

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Amyloid Activation Of Microglia

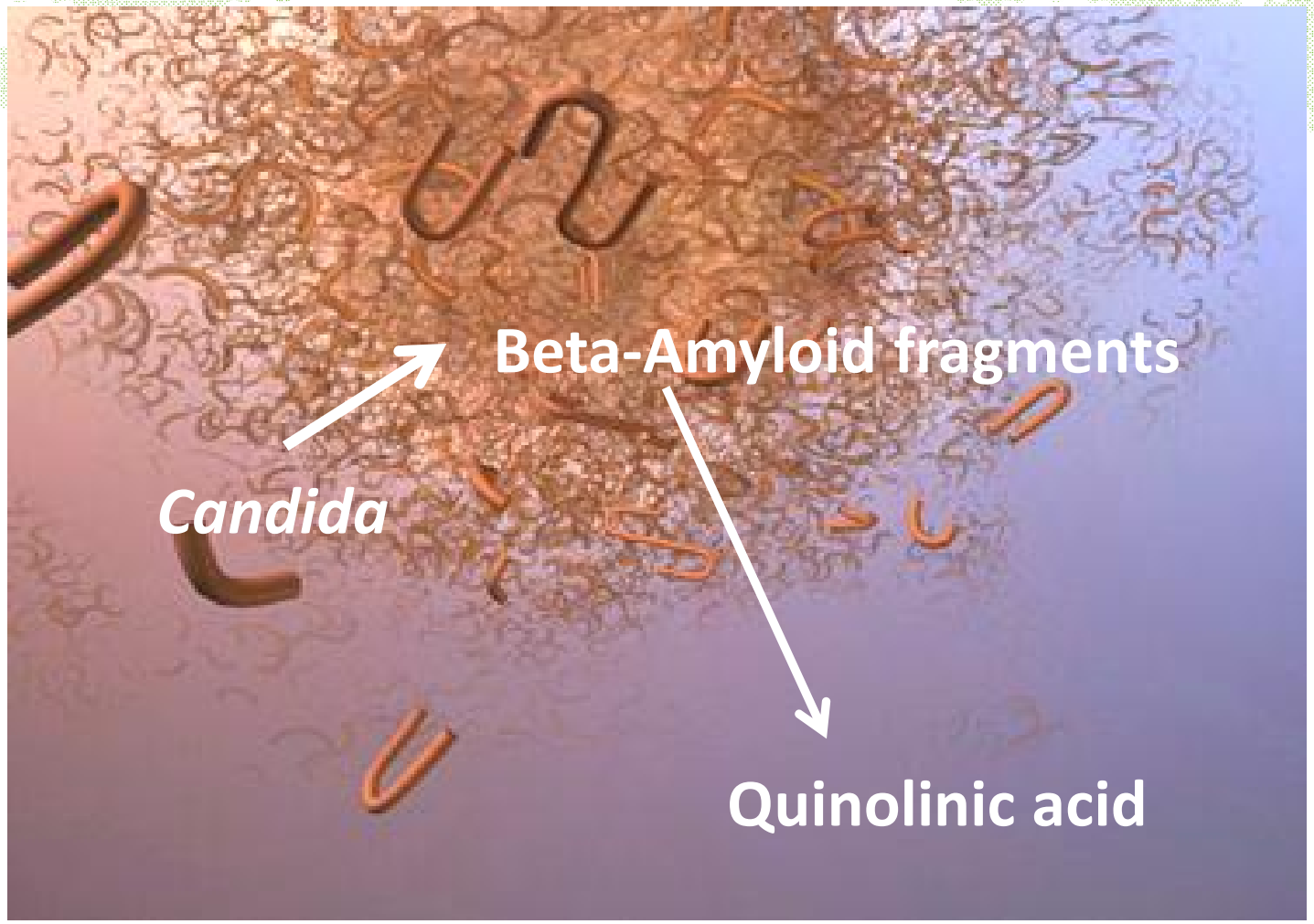
J Biol Chem. 2011 Feb 4;286(5):3693-706. Epub 2010 Oct 22.

Amyloid-beta protein oligomer at low nanomolar concentrations activates microglia and induces microglial neurotoxicity.

Maezawa I, Zimin PI, Wulff H, Jin LW.

Abstract

Neuroinflammation and associated neuronal dysfunction mediated by activated microglia play an important role in the pathogenesis of Alzheimer disease (AD). Microglia are activated by aggregated forms of amyloid-β protein (Aβ), usually demonstrated in vitro by stimulating microglia with micromolar concentrations of fibrillar Aβ, a major component of amyloid plaques in AD brains. Here we report that amyloid-β oligomer (AβO), at 5-50 nm, induces a unique pattern of microglia activation that requires the activity of the scavenger receptor A and the Ca(2+)-activated potassium channel KCa3.1. AβO treatment induced an activated morphological and biochemical profile of microglia, including activation of p38 MAPK and nuclear factor κB. Interestingly, although increasing nitric oxide (NO) production, AβO did not increase several proinflammatory mediators commonly induced by lipopolyliposaccharides or fibrillar Aβ, suggesting that AβO stimulates both common and divergent pathways of microglia activation. AβO at low nanomolar concentrations, although not neurotoxic, induced indirect, microglia-mediated damage to neurons in dissociated cultures and in organotypic hippocampal slices. The indirect neurotoxicity was prevented by (i) doxycycline, an inhibitor of microglia activation; (ii) TRAM-34, a selective KCa3.1 blocker; and (iii) two inhibitors of inducible NO synthase, indicating that KCa3.1 activity and excessive NO release are required for AβO-induced microglial neurotoxicity. Our results suggest that AβO, generally considered a neurotoxin, may more potently cause neuronal damage indirectly by activating microglia in AD.



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OPEN ACCESS Freely available online

PLoS one

The Alzheimer's Disease-Associated Amyloid β-Protein Is an Antimicrobial Peptide

Stephanie J. Soscia[1,2], James E. Kirby[3], Kevin J. Washicosky[1], Stephanie M. Tucker[1], Martin Ingelsson[4], Bradley Hyman[1,5], Mark A. Burton[6,7], Lee E. Goldstein[6,7], Scott Duong[3], Rudolph E. Tanzi[1,5]*, Robert D. Moir[1,5]

1 Genetics and Aging Research Unit, Mass General Institute for Neurodegenerative Disease and Department of Neurology, Massachusetts General Hospital, Charlestown, Massachusetts, United States of America, **2** Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, Massachusetts, United States of America, **3** Department of Pathology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States of America, **4** Department of Public Health/Geriatrics, Uppsala University, Uppsala, Sweden, **5** Harvard Medical School, Boston, Massachusetts, United States of America, **6** Molecular Aging and Developmental Laboratory, Photonics Center, College of Engineering, Boston University School of Medicine, Boston University, Boston, Massachusetts, United States of America, **7** Boston University Alzheimer's Disease Center, Boston University, Boston, Massachusetts, United States of America

Abstract

Background: The amyloid β-protein (Aβ) is believed to be the key mediator of Alzheimer's disease (AD) pathology. Aβ is most often characterized as an incidental catabolic byproduct that lacks a normal physiological role. However, Aβ has been shown to be a specific ligand for a number of different receptors and other molecules, transported by complex trafficking pathways, modulated in response to a variety of environmental stressors, and able to induce pro-inflammatory activities.

Methodology/Principal Findings: Here, we provide data supporting an *in vivo* function for Aβ as an antimicrobial peptide (AMP). Experiments used established *in vitro* assays to compare antimicrobial activities of Aβ and LL-37, an archetypical human AMP. Findings reveal that Aβ exerts antimicrobial activity against eight common and clinically relevant microorganisms with a potency equivalent to, and in some cases greater than, LL-37. Furthermore, we show that AD whole brain homogenates have significantly higher antimicrobial activity than aged matched non-AD samples and that AMP action correlates with tissue Aβ levels. Consistent with Aβ-mediated activity, the increased antimicrobial action was ablated by immunodepletion of AD brain homogenates with anti-Aβ antibodies.

Conclusions/Significance: Our findings suggest Aβ is a hitherto unrecognized AMP that may normally function in the innate immune system. This finding stands in stark contrast to current models of Aβ-mediated pathology and has important implications for ongoing and future AD treatment strategies.

Citation: Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, et al. (2010) The Alzheimer's Disease-Associated Amyloid β-Protein Is an Antimicrobial Peptide. PLoS ONE 5(3): e9505. doi:10.1371/journal.pone.0009505

Possible Effects of *Candida* in Variety of Diseases

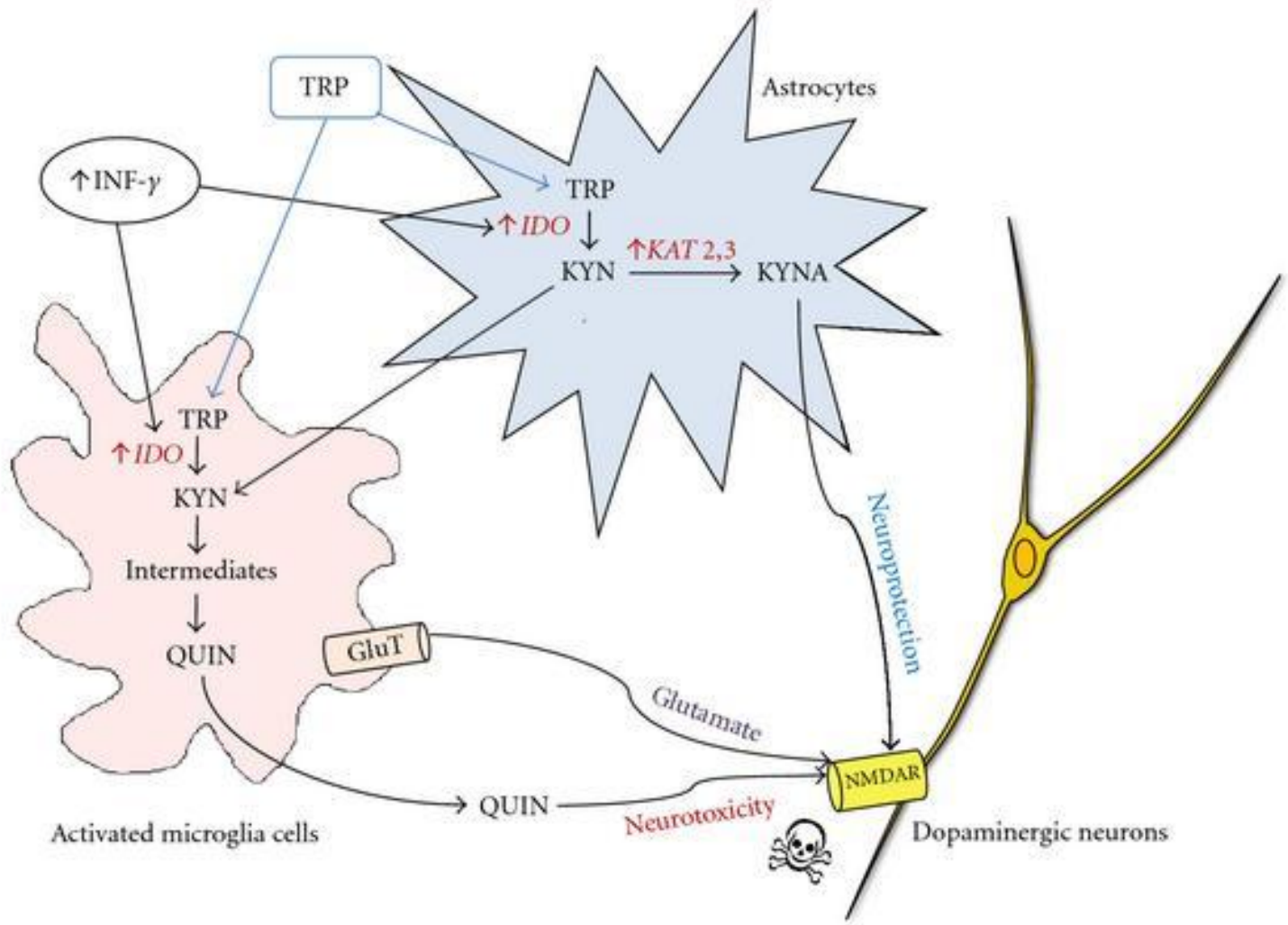
- *Candida* causes the production of beta-amyloid as an immune response.
- **Beta-amyloid is a major inducer of indoleamine oxygenase (IDO) which reduces tryptophan and depletes serotonin and increases neurotoxic quinolinic acid.**
- Brain fog and depression in disorders like chronic fatigue syndrome and fibromyalgia may be due to imbalances in serotonin, tryptophan, and quinolinic acid caused by action of beta-amyloid on IDO.

Neurotoxic Rec. 2000;2(2-3):139-55. Excitotoxicity of quinolinic acid: modulation by endogenous antagonists. Jhamandas KH, et.al

Quinolinic acid (QUIN), a product of tryptophan metabolism by the kynurenine pathway, produces excitotoxicity by activation of NMDA receptors. Focal injections of QUIN can deplete the biochemical markers for dopaminergic, cholinergic, gabanergic, enkephalinergic and NADPH diaphorase neurons, which differ in their sensitivity to its neurotoxic action. This effect of QUIN differs from that of other NMDA receptor agonists in terms of its dependency on the afferent glutamatergic input and its sensitivity to the receptor antagonists. The enzymatic pathway yielding QUIN produces metabolites that inhibit QUIN-induced neurotoxicity. The most active of these metabolites, kynurenic acid (KYNA), blocks NMDA and non-NMDA receptor activity. Treatment with kynurenine hydroxylase and kynureinase inhibitors increases levels of endogenous KYNA in the brain and protects against QUIN-induced neurotoxicity. Other neuroprotective strategies involve reduction in QUIN synthesis from its immediate precursor, or endogenous synthesis of 7-chloro-kynurenic acid, a NMDA antagonist, from its halogenated precursor. Several other tryptophan metabolites--quinaldic acid, hydroxyquinaldic acid and picolinic acid--also inhibit excitotoxic damage but their presence in the brain is uncertain. Picolinic acid is of interest since it inhibits excitotoxic but not neuroexcitatory responses. The mechanism of its anti-excitotoxic action is unclear but might involve zinc chelation. Neurotoxic actions of QUIN are modulated by nitric oxide (NO). Treatment with inhibitors of NO synthase can augment QUIN toxicity in some models of excitotoxicity suggesting a neuroprotective potential of endogenous NO. In recent studies, certain nitroso compounds which could be NO donors, have been reported to reduce the NMDA receptor-mediated neurotoxicity. The existence of endogenous compounds which inhibit excitotoxicity provides a basis for future development of novel and effective neuroprotectants.

Quinolinic Acid is a NMDA Receptor Stimulator

Kynurenic Acid Blocks NMDA Receptor Activity



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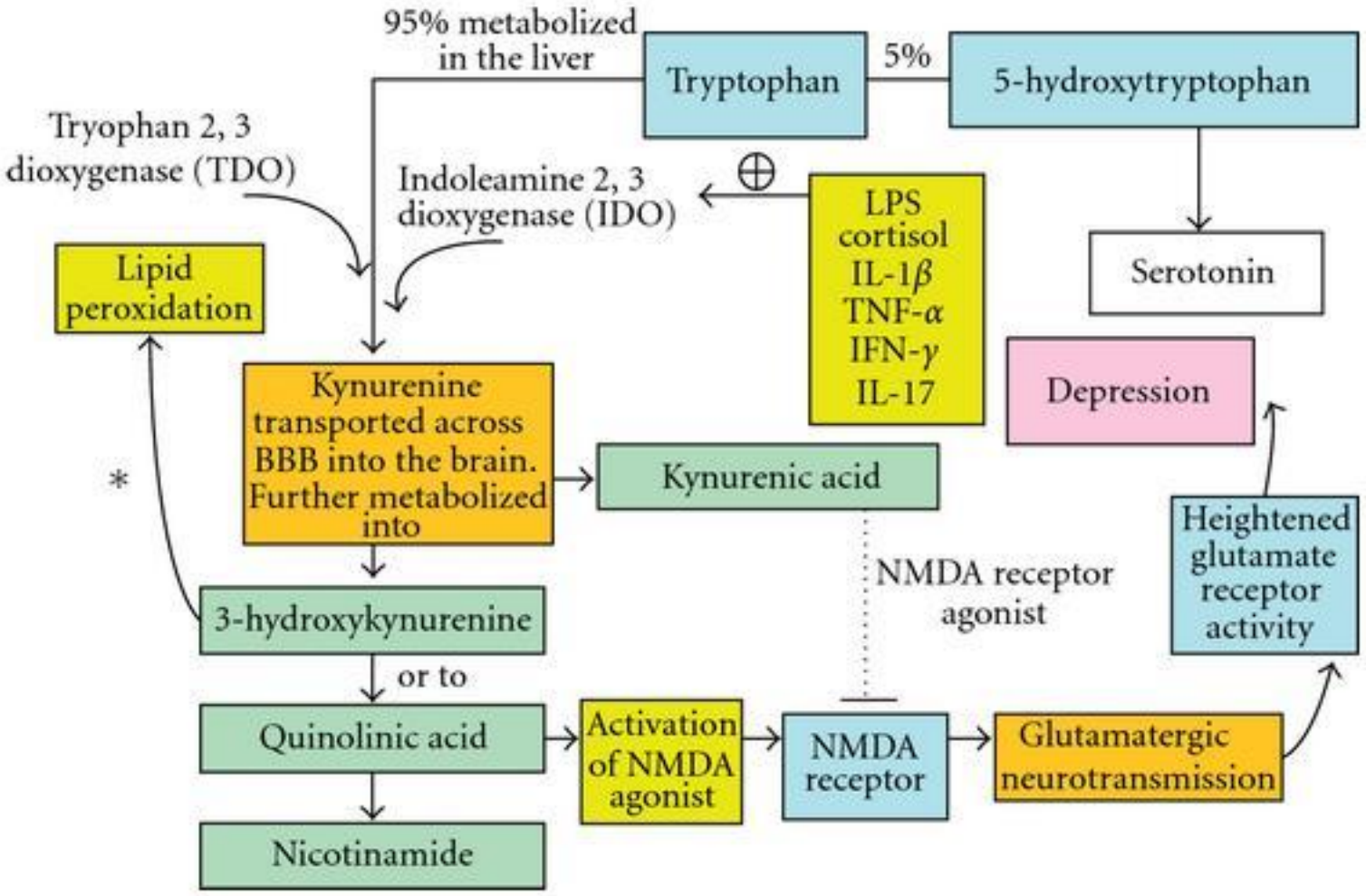

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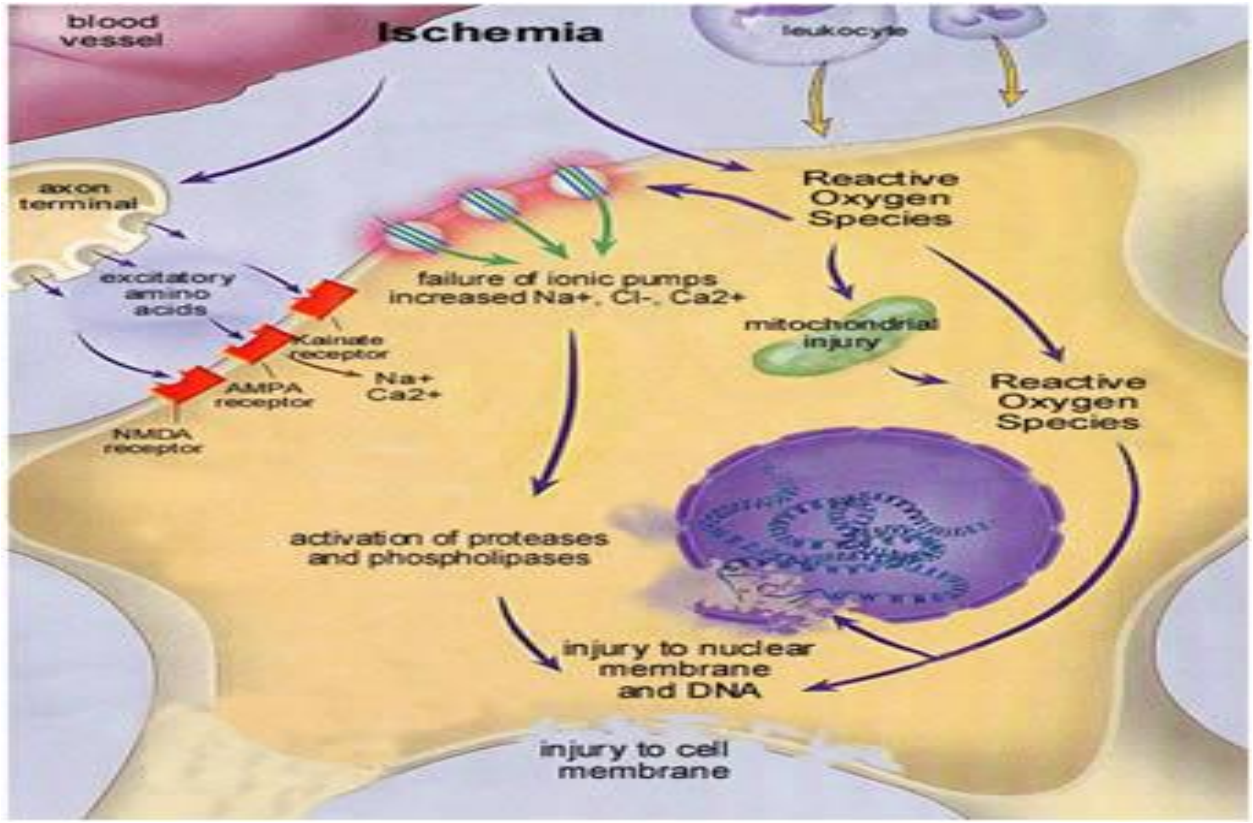
Receptor Hyper-Reactivity/ Stimulation



NMDA & other excitatory receptors

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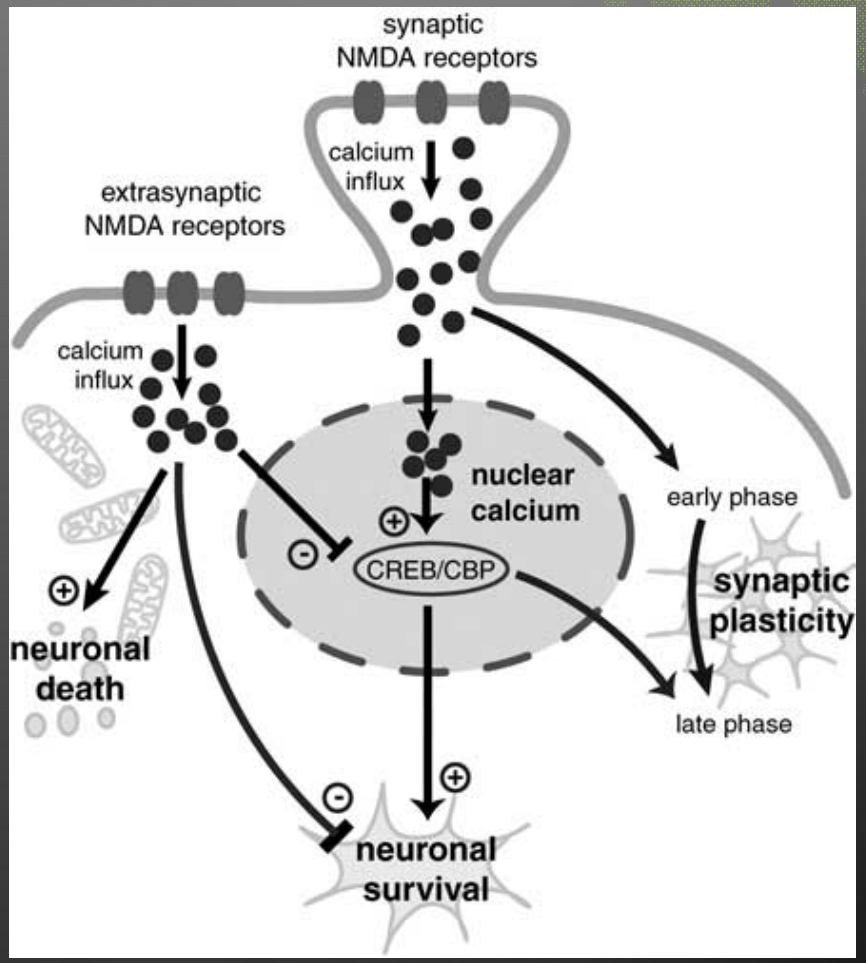
Namenda

Namenda (mematine):

- Approved for Alzheimer's Disease
- Blocks NMDA receptors
- In autism:
  - Increase language
  - Decreased aggression, tantrums
  - Decreased perseverations
  - Better mood
- Dosing:
  - 20mg for Alzheimer's patients
  - 5 to 10 mg for autistic kids
  - Starting at 2.5 to 5mg per day with a target dose being twice daily.
  - Side Effects – agitation, irritability
    - These could be related to ingredients
    - Dyes, propylene glycol



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L-Theanine and Lithium (nutritional)

Theanine

- Amino Acid found in Green Tea.
- Related to Glutamine – crosses the BBB.
- Increases GABA, as well as Serotonin and Dopamine.
- Affinity for NMDA, Kainate and AMPA receptors.
- Helps block glutamate excitotoxicity.

New Beginnings (www.nbnus.com):

- **100mg capsules – 1 to 6 capsule per day. Taken along with GABA 500mg – 1 to 6 per day can help with mood, aggression, etc.**

Lithium

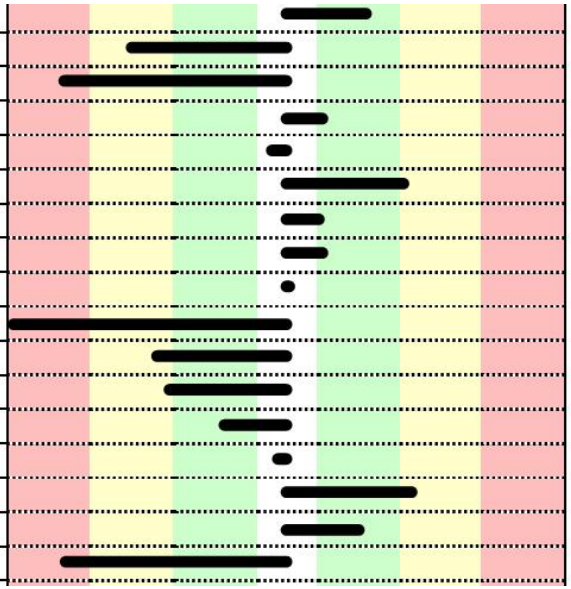
- Helps to regulate the NMDA receptor.
- Improves mood, cognitive performance, mental sharpness
- High Doses (carbonate – 300 to 1800mg) used for Bipolar Disorder.
- Elemental or Orotate Lithium – between 5 to 20mg helpful for brain health.

New Beginnings (www.nbnus.com):

- **Elemental Lithium (liquid) – 10 drops = 500mcg. Recommended daily dosing is 10 drops for adults and 3 to 5 drops for children.**

Low Lithium Commonly Found in Autism

Potassium	**(K)**	**160**	12- 200
Copper	**(Cu)**	**9.9**	11- 18
Zinc	**(Zn)**	**38**	100- 190
Manganese	**(Mn)**	**0.30**	0.10- 0.50
Chromium	**(Cr)**	**0.56**	0.43- 0.80
Vanadium	**(V)**	**0.12**	0.030- 0.10
Molybdenum	**(Mo)**	**0.095**	0.050- 0.13
Boron	**(B)**	**3.0**	0.70- 5.0
Iodine	**(I)**	**0.57**	0.25- 1.3
Lithium	**(Li)**	**0.004**	0.007- 0.020
Phosphorus	**(P)**	**145**	150- 220
Selenium	**(Se)**	**0.68**	0.70- 1.1
Strontium	**(Sr)**	**0.22**	0.16- 1.0
Sulfur	**(S)**	**47800**	45500- 53000
Cobalt	**(Co)**	**0.026**	0.004- 0.020
Iron	**(Fe)**	**14**	7.0- 16
Germanium	**(Ge)**	**0.019**	0.030- 0.040



Integrative Medicine Academy

www.StopCallingItAutism.org

Dedicated to investigating the diagnosis and treatment of medical issues related to neurological inflammation, immune dysfunction and microglia activation in autism.

Ibuprofen and Autism Intervention – ***to be discussed during the Question and Answer session for Module #12***

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The History of the Tryptophan Controversy

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Tryptophan versus 5-HTP

- Tryptophan formerly banned in USA without prescription for many years due to toxicity. Impurity?
- 5-Hydroxytryptophan (5-HTP) was, and still is available as a supplement.
- 5-HTP preferable because doesn't produce neurotoxic quinolinic acid.
- Causes drowsiness so may best be given at bedtime (100mg –300 mg) adult.
- Reduce if person still sleepy or sluggish next day or if side effects occur – *bad dreams*.
- Tryptophan most common uses: ADD, PMS, depression, high protein diet (Atkins), or obesity.

History of Tryptophan and Eosinophilia-Myalgia Syndrome (EMS)

- 1989: large outbreak of eosinophilia myalgia syndrome
- 1500 cases of permanent disability and 37 deaths
- Connection with contaminated lot?
- 1991: OTC tryptophan banned by U.S. FDA, but prescription sales allowed.
- 2002: OTC sales allowed again by FDA
- **FDA Statement:** *"Based on the scientific evidence that is available at the present time, we cannot determine with certainty that the occurrence of EMS in susceptible persons consuming L-tryptophan supplements derives from the content of L-tryptophan, an impurity contained in the L-tryptophan, or a combination of the two in association with other, as yet unknown, external factors."*

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Silver RM, et al. "Scleroderma, fasciitis, and eosinophilia associated with the ingestion of tryptophan." *N Engl J Med*. 1990 Mar 29;322(13):874–81

- An association between past tryptophan use and a syndrome of scleroderma-like skin abnormalities, fasciitis, and eosinophilia had been recognized in the USA .
- Plasma levels of L-kynurenine and quinolinic acid, which are metabolites of tryptophan, were significantly higher in four patients with active disease than in three patients studied after eosinophilia had resolved or in five normal subjects ($P<0.001$).
- This illness resembles eosinophilic fasciitis and probably represents one aspect of the recently reported eosinophilia-myalgia syndrome.
- The development of the syndrome may result from a confluence of several factors, including the ingestion of tryptophan, exposure to agents that activate indoleamine-2,3-dioxygenase, and possibly, impaired function of the hypothalamic-pituitary-adrenal axis.

Ito J, Hosaki Y, Torigoe Y, Sakimoto K (1992). "Identification of substances formed by decomposition of peak E substance in tryptophan". *Food Chem. Toxicol.* 30 (1): 71–81

"It eventually became clear that the cause had not been the tryptophan itself, but rather that flaws in Showa Denko's 1980s manufacturing process (long since corrected) had allowed trace impurities to contaminate these batches, and those impurities were in turn responsible for the 1989 EMS outbreak."

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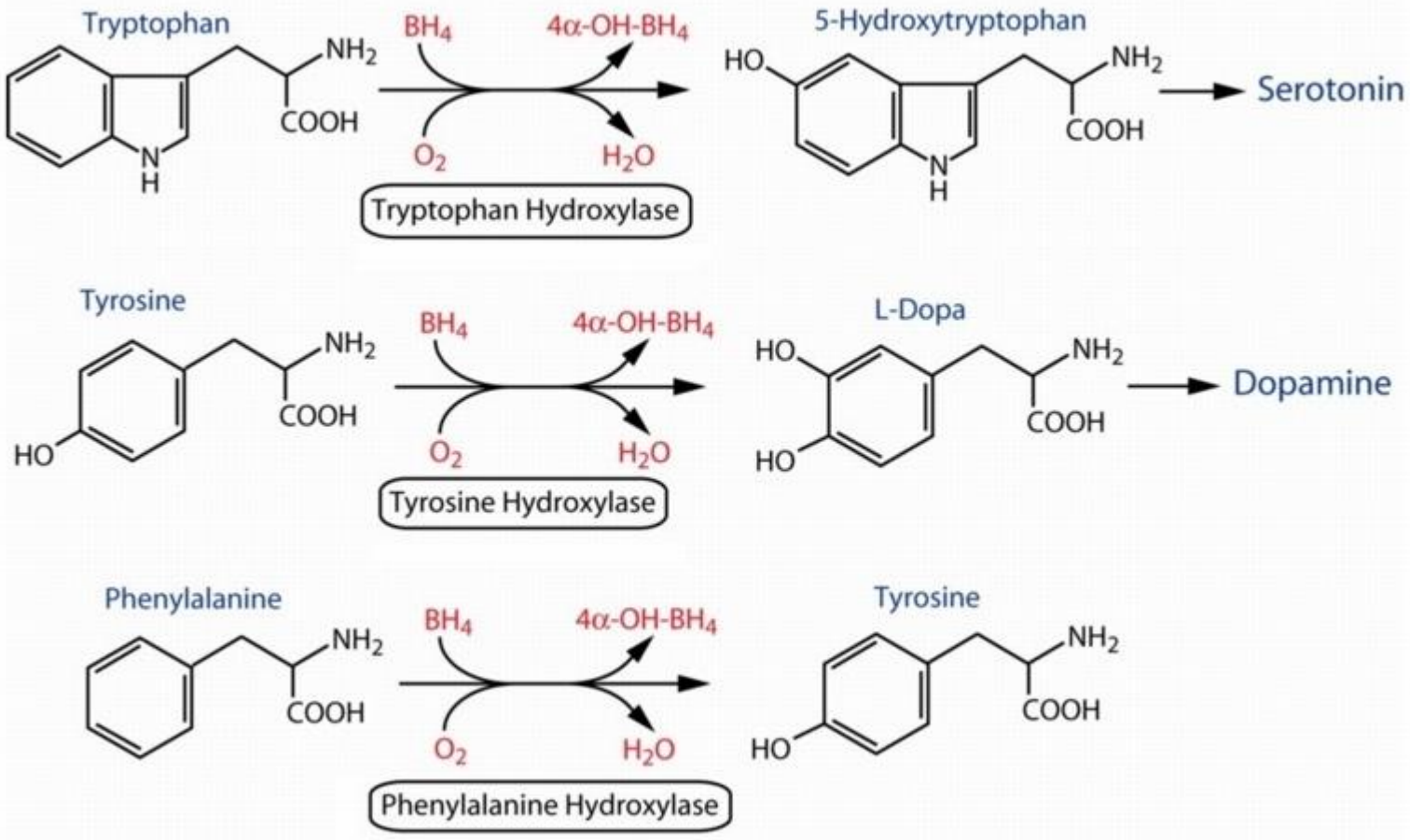
Smith MJ, Garrett RH. "A heretofore undisclosed crux of Eosinophilia-Myalgia Syndrome: compromised histamine degradation." *Inflamm Res*. 2005;54(11):435–50

- Perhaps 60,000 consumers were seriously afflicted by eosinophilia with myalgia in the 1980s.
- 400 direct deaths had been recorded
- Incriminated case lots from Japanese company had analytical purity of 98.5%–99.6%.
- 3 official EMS cases in the United Kingdom that were associated with a Merck pharmaceutical product containing L-tryptophan (Optimax) – *not related to Japanese product.*
- An analogous survey in Ireland disclosed 5 cases of eosinophilia linked to Optimax.
- The Merck product was completely unrelated to the Japanese "contaminated" product.

Noakes R, et al. "Is the L-tryptophan metabolite quinolinic acid responsible for eosinophilic fasciitis?" *Clin Exp Med.* 2006 Jun;6 (2):60–4

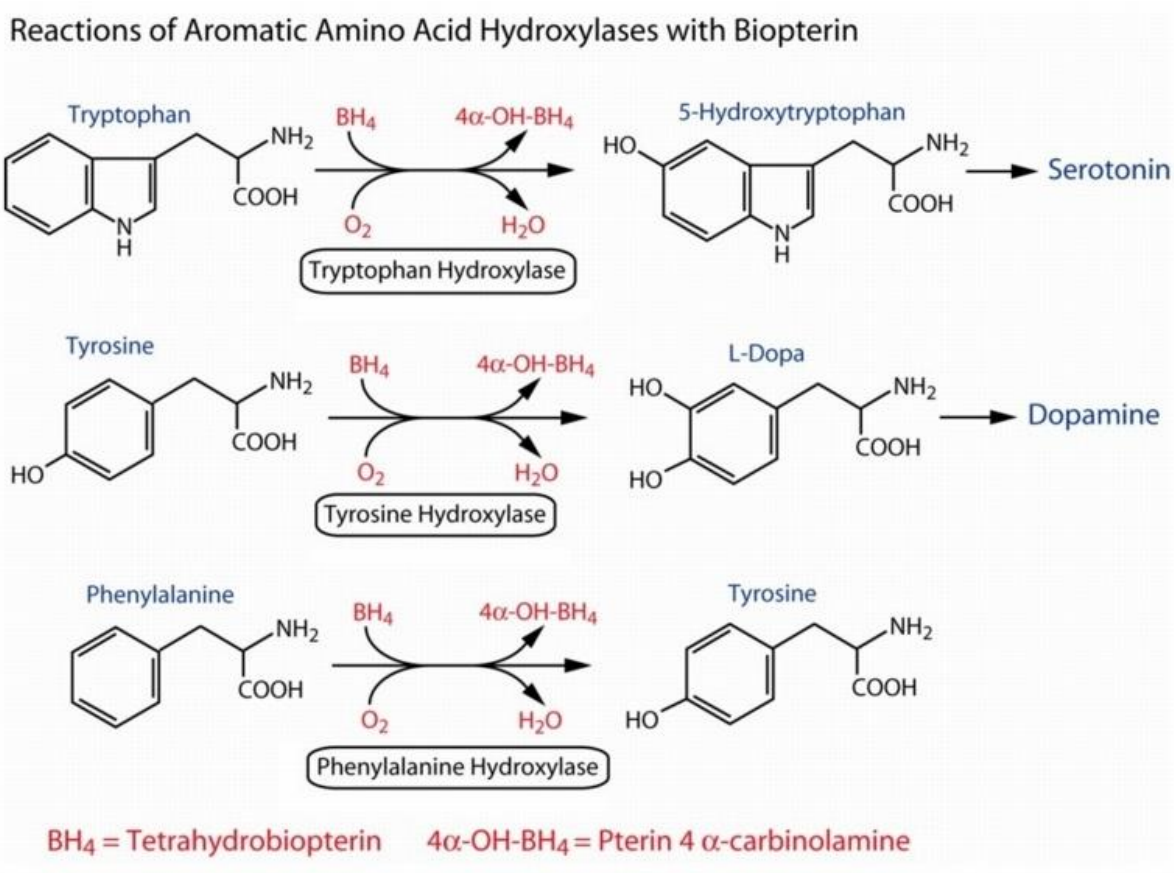
- Author (R. Noakes) received a series of subcutaneous injections of quinolinic acid (1200 mg over a 1-month period).
- Over the 1-month period the eosinophil count rose from $0.3x10^9$/l to $0.8x10^9$/l before falling to $0.4x10^9$/l approximately 5 weeks later.
- Stained sections showed a mixed infiltrate of eosinophils and neutrophils in tissue histology samples.
- Our results suggest that quinolinic acid may play a role in cutaneous eosinophilic disorders.
- **<u>Significance</u>: "tryptophan is major source of quinolinic acid, while 5-hydroxytryptophan is not and may be a much better supplement that does not cause eosinophilia myalgia."**

Reactions of Aromatic Amino Acid Hydroxylases with Biopterin



BH_4 = Tetrahydrobiopterin 4α-OH-BH_4 = Pterin 4 α-carbinolamine

5-HTP is committed towards the Serotonin pathway. It cannot go backwards to Quinolinic Acid.



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Treatment Options for Elevated Quinolinic Acid

Treatment Options for High Quinolinic Acid

1. Address underlying infections and other stressors as best as possible:
   - *Organic Acids Test*
   - *Stool Pathogen Testing, i.e. CDSA*
   - *Viral Testing – IgG & IgM: herpes viruses, i.e. I, II, VI, CMV, EBV.*
2. Foundational support nutrients (vitamins, minerals, essential fatty acids), including antioxidants:
   - Can add other antioxidants such as curcumin (*and other polyphenols such as green tea extract*), glutathione (and support products, i.e. broccoli extracts), N-acetyl-cysteine, vitamin E, Resveratrol, etc.

Resveratrol

Anti-inflammatory activities of resveratrol in the brain: role of resveratrol in microglial activation

Zhang F, Liu J, Shi JS. Shanghai University of Traditional Chinese Medicine, Shanghai 200071, China

Eur J Pharmacol. 2010 Jun 25;636(1-3):1-7

Neuroinflammation is an important contributor to pathogenesis of neurological disorders, with microglial activation as a hallmark of neuroinflammation. Microglia serve the role of immune surveillance under normal conditions, but after brain damage or exposure to inflammation, microglia are activated and secrete pro-inflammatory and neurotoxic mediators. Sustained production of these factors contributes to neuronal damage. Therefore, inhibition of microglia-mediated neuroinflammation may become a promising therapeutic target for neurological disorders. Resveratrol, a non-flavonoid polyphenol rich in red wine and grapes, has beneficial health effects from its antioxidant, anticancer and anti-inflammatory properties. Recently, resveratrol has been shown to protect against various neurological disorders in experimental models, including brain ischemia, seizures, and neurodegenerative disease models. This minireview summarized the anti-inflammatory activities of resveratrol in the brain from both in vivo and in vitro studies, and highlighted the inhibition of activated microglia as a potential mechanism of neuroprotection.

The release of various pro-inflammatory factors, the production of reactive oxygen species, and the activation of signal pathways leading to neuroinflammation were discussed in relation to microglial activation. Taken together, microglia are an important target for anti-inflammatory activities of resveratrol in the brain.

What is Resveratrol?

- A compound found in red wine and grapes
- Shown to be helpful against seizures, oxidative stress, and neurodegenerative disorders.
- **Helps against 'Microglial Activation'**
- 500mg to 1000mg daily can be helpful against brain inflammation alone or in conjunction with other natural remedies.

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Liquid Glutathione

Lipoceutical Glutathione:

- Kids: *¼ to ½ teaspoon per 30lbs body weight twice daily.*
- Adults: *1 to 2 teaspoons daily.*
- *Available from New Beginnings Nutritionals – www.nbnus.com*

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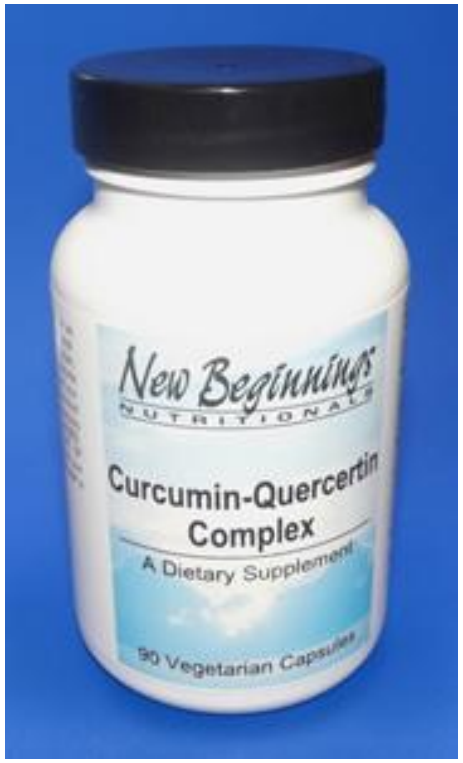
Curcumin (*and Quercetin*)



2 to 4 capsules daily

1 to 2 capsules daily

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Treatment Options for High Quinolinic Acid in Autism

Niacinamide

- One of the two principal forms of Vitamin B3 (niacin).
- Niacinamide is the biologically active form of niacin.
- Assists in ATP production
- 500mg to 1000mg+ daily
- No flushing
- Garlic and melatonin – *also reported to help with elevated quinolinic acid.*

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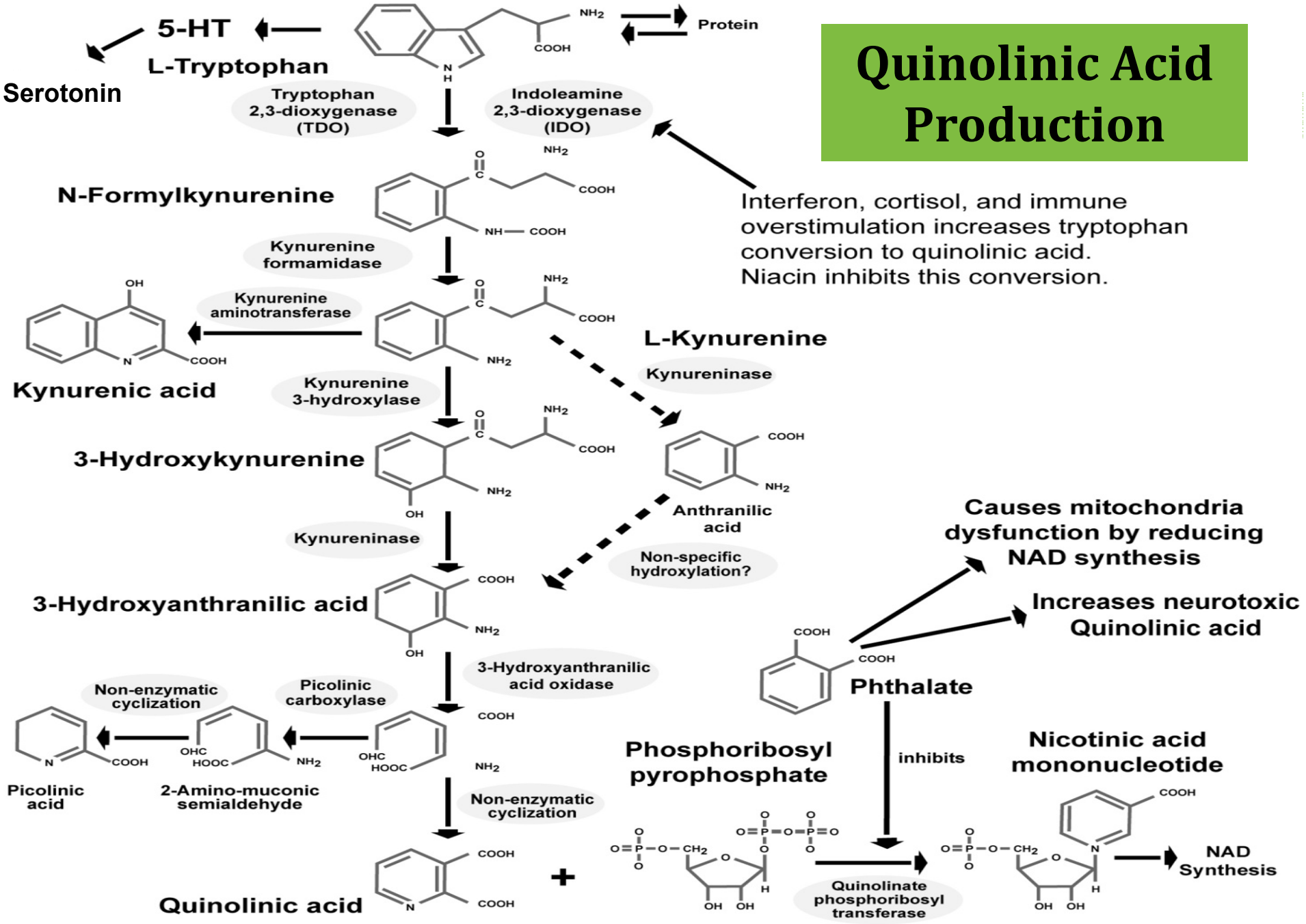
Quinolinic Acid Production



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MOLECULAR PHARMACOLOGY, 43:976-981

Stimulation by Glutamate Receptors of Arachidonic Acid Release Depends on the Na^+/Ca^{2+} Exchanger in Neuronal Cells

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SUMMARY

In primary cultures of striatal neurons, stimulation of *N*-methyl-D-aspartic acid (NMDA) receptors or associative activation (but not separate activation) of (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and metabotropic glutamate receptors (mGluR) strongly increased arachidonic acid (AA) release via activation of phospholipase A_2 (PLA_2). Depolarizing agents, such as veratridine, were as potent as NMDA in stimulating AA release. However, increasing the intracellular Ca^{2+} concentration via voltage-sensitive Ca^{2+} channels did not result in a significant stimulation of PLA_2. Substitution of sodium by lithium, a monovalent cation that does not participate in the Na^+/Ca^{2+} exchanger activity but permeates ionotropic glutamate receptor channels, blocked AA release induced by veratridine or AMPA plus mGluR agonists. It also reduced the NMDA-induced AA release, to a lesser extent. The contribution of the Na^+/Ca^{2+} exchanger to the activation of PLA_2 after veratridine, NMDA receptor, or AMPA receptor plus mGluR stimulation was confirmed by using a selective inhibitor of the Na^+/Ca^{2+}exchanger.

Phospholipase A2 and the connection to cellular inflammation and neurological problems – *discussed during the Question and Answers Module #12*

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Module #13

Topic

- Troubleshooting Aberrant Behaviors, Common Problems and Various Clinical Issues in Autism – A to Z:
  - *Troubleshooting common problems, i.e. aberrant behaviors seen with individuals on the autism-spectrum.*
  - *Suggestions for intervention, including testing, supplements, etc. that can help overcome certain behavioral and physical issues.*
  - *Understanding that not every problem is related to a biomedical issue.*

Bonus Lecture (Q&A session) - Phospholipase A2 and Ibuprofen Trial in Autism

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Thank You

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