Biology of D-Amino Acid Oxidase (DAAO)

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D-Amino Acid Oxidase (DAAO)

- Discovered more than 70 years ago by Krebs after he observed that amino acids belonging to the “d-series were deaminated much more rapidly than the natural isomericides” by fresh pig kidney and liver homogenates;
- A Flavoenzyme with a monomer of 347 amino acids containing one noncovalently bound FAD with MW of approximately 40 kDa.
DAAO Expression and Distribution in Mammals

- Found in numerous eukaryotic organisms, including yeasts, fungi, insects, amphibians, reptiles, birds and mammals;
- Expressed in the kidneys, liver and central nervous system including hindbrain, cerebellum and spinal cord.

Wang et al., Current Drug Metab., 2012
DAAO Enzymatic Activity

- Catalyzes with a strict stereospecificity the oxidative deamination of D-amino acids to give α-keto acids and ammonia; oxidized FAD is reduced leading to production of hydrogen peroxide from oxygen;
- Substrates: neutral and polar D-amino acids such as endogenous D-serine, D-dopa and D-alanine, or exogenous D-nitroarginine serve as substrates.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Km (mmol/L)</th>
<th>Vmax</th>
<th>Kcat/Km</th>
<th>V*</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Ala</td>
<td>9.15 ± 0.69</td>
<td>100</td>
<td>78</td>
<td>100</td>
</tr>
<tr>
<td>D-NNA</td>
<td>26.53 ± 5.03</td>
<td>11</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>D-Phe</td>
<td>5.22 ± 0.26</td>
<td>72</td>
<td>100</td>
<td>94</td>
</tr>
<tr>
<td>D-Leu</td>
<td>6.81 ± 1.16</td>
<td>47</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td>D-Arg</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>4</td>
</tr>
<tr>
<td>D-NAME</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>6</td>
</tr>
</tbody>
</table>

Wang et al., Current Drug Metab., 2012
Functions of DAAO in Mammals

- D-amino acid metabolism, nutrition and toxicity
  - Utilize D-phenylalanine in place of its L-isomer;
  - D-Serine-induced nephrotoxicity;
- Component of an antibacterial system in neutrophils;
- Novel schizophrenia drug targets
  - The NMDA receptor hypofunction theory of schizophrenia;
- Chronic pain and morphine tolerance
DDY/DAAO\(^{-/-}\) Mice
(from Prof. Konno’s Lab, Japan)

An autosomal missense mutation (Gly-181→Arg) in the DAAO gene leading to a loss of DAAO enzymatic activity

Xin et al., Chem. Biodivers., 2010.
DAAO Inhibitors

- Benzoic Acid
- CBIO (5-chlorobenzo[d]isoxazol-3-ol)
- AS057278 (5-methyl-3-pyrazole-carboxylic acid)
- NPCA (4-nitro-3-pyrazole carboxylic acid)
- Compound 8 (sc-203909) ([4H]-thieno[3,2-b]pyrrole-5-carboxylic acid)
- Compound 2 (3-hydroxyquinolin-2-[1H]-one)

Gong et al., J Phar Exp Ther, 2011
shRNA/DAAO and siRNA/DAAO Targeting Spinal DAAO Expression

Chen et al., BBRC, 2012
Chiral Inversion of D-Amino Acids
L-Arginine/Nitric Oxide/cGMP Pathway

Endotoxin Cytokines → L-arg → Shearing Forces

Acetylcholine Bradykinin Substance-P Insulin

Endothelial Cell → NO + citrulline

Target Cell → NO → GC → cGMP

GTP → G-cyclase → cGMP → G-kinase (Relaxation)

Endothelial Cell → L-Arginine → NOS → NO

NO → O2 → L-Citrulline

Ca2+ → L-NMMA L-NAME

HbO2

GTP → G-cyclase → cGMP → G-kinase (Relaxation)
## NOS Inhibitors: Potency and Selectivity

<table>
<thead>
<tr>
<th></th>
<th>eNOS</th>
<th>nNOS</th>
<th>iNOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-NMMA</td>
<td>316 nM</td>
<td>251 nM</td>
<td>3 μM</td>
</tr>
<tr>
<td>L-NNA</td>
<td>91 nM</td>
<td>47 nM</td>
<td>3 μM</td>
</tr>
<tr>
<td>L-NAME</td>
<td>2 μM</td>
<td>758 nM</td>
<td>32 μM</td>
</tr>
<tr>
<td>L-NIO</td>
<td>2 μM</td>
<td>1 μM</td>
<td>2 μM</td>
</tr>
<tr>
<td>DIP</td>
<td></td>
<td>3 μM</td>
<td></td>
</tr>
<tr>
<td>7-NI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminoguanidine</td>
<td>190 nM</td>
<td></td>
<td>40 nM</td>
</tr>
<tr>
<td>1400W</td>
<td>50 μM</td>
<td>2 μM</td>
<td>7 nM</td>
</tr>
<tr>
<td>ONO-1714</td>
<td>19 nM</td>
<td></td>
<td>2 nM</td>
</tr>
</tbody>
</table>
Pressor Responses to L-NNA and D-NNA Are Blocked by L-Arginine But Not D-Arginine

Wang et al., Current Drug Metab., 2012
Hypothesis

• In vitro-IC$_{50}$: D-NNA/L-NNA: 400/1; In vivo: ED$_{50}$: D-NNA/L-NNA: 2/1
• Rise phase $T_{1/2}$: 27/5
• Effects of L-NNA and D-NNA were blocked by L-arginine

Wang et al., J. Pharmacol. Exp. Ther., 1993
Chiral Separations of L-NNA and D-NNA

- Chiral ligand exchange chromatography
- Capillary electrochromatography, CEC
Unidirectional Conversion of D-NNA after Administration

- Drug-free plasma
- 60 min after D-NNA
- 60 min after L-NNA
Plasma D-NNA/L-NNA after L-NNA and D-NNA in Rats

Xin et al., J. Pharmacol. Exp. Ther., 2005
Pressor and Conversion Responses to D-NNA in Rats

Wang et al., J. Pharmacol. Exp. Ther., 1999
Chiral Inversion of D-NNA in Tissue Homogenates Incubated for One Hour

- Heart
- Lungs
- Liver
- Kidneys
Kidney Ligation Blocks Chiral Inversion of D-NNA

1: L-NNA
2: D-NNA.

- Bikidney-ligation
- Unikidney-ligation
- Sham operation
- Control

△ vehicle response in sham-operated rats
▲ D-NNA in sham-operated rats
□ D-NNA in unilateral kidney-ligated rats
■ D-NNA in bilateral kidney-ligated rats

Xin et al., J. Pharmacol. Exp. Ther., 2005
Correlation Between DAAO Activity and D-NNA Chiral Inversion

Xin et al., J. Pharmacol. Exp. Ther., 2005
Inhibitory Effects of Benzoate on Renal DAAO Activity and D-NNA Conversion

Xin et al., Drug Metab. Disp., 2007
Effects of Benzoate on Pressor Responses to D-NNA and L-NNA

B

\[ \Delta MAP \text{ (mmHg)} \]

A

\[ \Delta MAP \text{ (mmHg)} \]

Xin et al., J. Pharmacol. Exp. Ther., 2005

- □ Benzoate+vehicle (n = 3)
- ■ Vehicle+D-NNA (n = 3)
- △ Benzoate (80 mg/kg)+D-NNA (n = 4)
- ▲ Benzoate (400 mg/kg)+D-NNA (n =6)
- ○ Vehicle+L-NNA (n =3)
- ● Benzoate (400 mg/kg)+L-NNA (n = 3)
Plasma L-NNA/D-NNA after L-NNA and D-NNA Administration in Benzoate-Pretreated Rats

Xin et al., Drug Metab. Disp., 2007
Plasma L- and D-NNA after i.v. D-NNA in normal Swiss mice, ddY/DAAO\(^{+/+}\) mice and ddY/DAAO\(^{-/-}\) mice

Xin et al., Chem. Biodivers., 2010
Chiral Inversion of D-NNA in Mouse and Rat Tissue Homogenates with/without DAAO Enzymatic Activity

Xin et al., Chem. Biodivers., 2010
Two-Step Hypothesis for D-NNA Chiral Inversion

- D-NNA leading to α-keta acid by DAAO;
- α-Keta acid leading to L-NNA by unknown aminotransferase(s)

Wang et al., Current Drug Metab., 2012
Production of L-NNA and Pressor Responses by N^G-Nitro-Guanidino-2-Oxopetanoic Acid in Conscious Rats Pretreated with Benzoate

Xin et al., Drug Metab. Disp., 2007
Summary and Conclusions

• Blockade effect of D-NNA on NO synthesis is due to its unidirectional chiral inversion;
  – Unidirectional chiral inversion is discovered as a novel and common metabolism pathway for D-amino acids: D-leucine, D-dopa, D-phenylalanine, etc.

• D-NNA’s chiral inversion occurs mainly (80%) in kidneys;

• DAAO plays an essential role on D-NNA conversion. However, the role of DAAO is not sufficient, the role of the 2nd enzyme as unidentified transaminase(s) on D-NNA conversion is suggested and under investigation;

• Chiral inversion plays significant role in metabolism, nutrition and biological activities of D-amino acid drugs.
Chronic Pain, Morphine Analgesic Tolerance and Hyperalgesia
Etiology of Neuropathic Pain

Peripheral or central nervous system tissue damage (ectopic and persistent discharge) or from altered processing of pain in the CNS – Central Sensitization
Morphine Analgesic Tolerance and Hyperalgesia

- Morphine is a potent opiate analgesic, acting directly μ-opioid receptor on the CNS to relieve pain;
- Morphine is regarded as the gold standard or benchmark of analgesics used to relieve severe pain and suffering;
- Tolerance to analgesia: effectiveness lessens quickly and significantly with repeated doses. Approx. 40%, as high as 2,000 mg/dose;
- As tolerance develops, increased doses are necessary for equivalent relief or hyperalgesia;
- High doses bring a variety of severe complications
  - Oversedation;
  - Reduced physical activity;
  - Respiratory depression;
  - Constipation;
  - Strong potential for addiction.
Overdose deaths have quadrupled since 1999
Central sensitization: up-regulation of the "wide dynamic range" second-order neuron in the spinal cord leading to non-painful stimuli perceived as pain.
Role of Astrocytes in Chronic Pain, and Morphine Analgesic Tolerance and Hyperalgesia
Specific Expression of DAAO in Astrocytes but not Microglia or Neurons in the Spinal Dorsal Horn

Gong et al., Anesthesiology, 2014
Spinal DAAO Is Upregulated During Chronic Pain Development

Zhao et al., J. Pharmacol. Exp. Ther., 2010
Huang et al., Amino Acids, 2012
Spinal DAAO Is Upregulated Accompanying Astrocyte Proliferation After Morphine Tolerance

Gong et al., Anesthesiology, 2014
DAAO Gene Mutation Inhibits Formalin-Induced Tonic Pain

Zhao et al., Cell. Mol. Neurobiol., 2008
DAAO Inhibitors Block Formalin-Induced Tonic Pain but not Acute Nociception

Gong et al., J Phar Exp Ther, 2011
shRNA/DAAO and siRNA/DAAO Inhibit Formalin-Induced Tonic Pain

Chen et al., BBRC, 2012
CBIO Blocks Mechanical Allodynia in the Rat Bone Cancer Pain Model

Huang et al., Amino Acids, 2012
siRNA/DAAO Blocks Mechanical Allodynia in the Rat Bone Cancer Pain Model

Huang et al., Amino Acids, 2012
SUN Blocks Mechanical Allodynia and Heat Hyperalgesia in Neuropathic Rats

Hopkins (Sunovion) et al., J Phar Exp Ther, 2013
SUN Blocks Spontaneous Activity and Mechanically Evoked Responses of Wide Dynamic Range Neurons in the Dorsal Horn in SNL Rats

Hopkins (Sunovion) et al., J Phar Exp Ther, 2013
DAAO Inhibitors Block REM Sleep Deprivation-Induced Mechanical Allodynia

Wei et al., Pharmacol. Biochem. Behav., 2013
CBIO Interacts with Morphine Analgesia in An Additive Manner

Gong et al., Neuropharmacology, 2012
CBIO Does Not Induce Analgesic Self-Tolerance in the Formalin Test

Gong et al., Neuropharmacology, 2012
DAAO Inhibitors and Gene Silencers Prevent Morphine Tolerance in the Formalin Test

Gong et al., Anesthesiology, 2014
CBIO Prevents Morphine Tolerance in the Bone Cancer Pain Model

Huang et al., Amino Acids, 2012
DAAO Inhibitors Reverse Morphine Analgesic Tolerance in the Formalin Test

Gong et al., Anesthesiology, 2014
DAAO Inhibitors Reverse Established Morphine Tolerance in the Hot-Plate Test

Gong et al., Anesthesiology, 2014
CBIO Prevents and Reverses Morphine Hyperalgesia

CBIO Prevents Chronic Morphine-Increased Spinal GFAP, IL-1β, IL-6-α and TNF-α Expression

Role of Spinal DAAO/H$_2$O$_2$ in Chronic Pain, and Morphine Analgesic Tolerance

The NMDA receptor hypofunction hypothesis of schizophrenia
Time-Courses of Formalin-Induced Pain Behavior and Spinal Hydrogen Peroxide Level in Mice

Lu et al., Br. J. Pharmacol., 2012
CBIO Blocks Spinal $\text{H}_2\text{O}_2$ Production Following Formalin Injection or Chronic Morphine Treatment

Lu et al., Br. J. Pharmacol., 2012

Gong et al., Anesthesiology, 2014
Hydrogen Peroxide Scavenger PBN and Catalyst Catalase Inhibit Formalin-Induced Tonic Pain

Lu et al., Br. J. Pharmacol., 2012
Hydrogen Peroxide Scavenger PBN and Catalyst Catalase Block Morphine Analgesic Tolerance

Gong et al., Anesthesiology, 2014
Hydrogen Peroxide Scavenger PBN and Catalyst Catalase Reverse Morphine Hyperalgesia

D-Serine Fails to Prevent Morphine-Hyperalgesia

Summary and Conclusion

• DAAO is restrictedly expressed in astrocytes in the spinal dorsal horn;

• Mutation and knockdown of DAAO gene, and blockade of DAAO activity lead to specific analgesia in chronic pain, and blockade of morphine tolerance and hyperalgesia: “one stone, two birds”;

• With hydrogen peroxide synthesis involved, spinal DAAO may be a potential target molecule for the treatment of chronic pain, and morphine tolerance and hyperalgesia;

• Drug companies have selected DAAO inhibitors as preclinical development candidates, now in early clinical investigation.
Thank You for Your Attention!