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REVIEW ARTICLE

D-Amino acids in the brain and mutant rodents lacking D-amino-acid oxidase activity

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Abstract D-Amino acids are stereoisomers of L-amino acids. They are often called unnatural amino acids, but several D-amino acids have been found in mammalian brains. Among them, D-serine is abundant in the forebrain and functions as a co-agonist of NMDA receptors to enhance neurotransmission. D-Amino-acid oxidase (DAO), which degrades neutral and basic D-amino acids, is mainly present in the hindbrain. DAO catabolizes D-serine and, therefore, modulates neurotransmission. In the brains of mutant mice and rats lacking DAO activity, the amounts of D-serine and other D-amino acids are markedly increased. Mutant mice manifested behavioral changes characteristic of altered NMDA receptor activity, likely due to increased levels of D-serine. D-Serine and DAO have been demonstrated to play important roles in cerebellar development and synaptic plasticity. They have also implicated in amyotrophic lateral sclerosis and pain response. There have also been several lines of evidence correlating DAO with schizophrenia. Taken together, the experiments indicate that D-amino acids and DAO have pivotal functions in the central nervous system.

Keywords D-Amino-acid oxidase · D-Serine · Mouse · Rat · NMDA receptor · Neuropsychological diseases

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Abbreviations

ACTH	Adrenocorticotropic hormone
ALS	Amyotrophic lateral sclerosis
CBIO	5-Chloro-benzo[*d*]isoxazol-3-ol
CNS	Central nervous system
DAO	D-Amino-acid oxidase
EPSC	Excitatory postsynaptic current
LTD	Long-term depression
LTP	Long-term potentiation
MK-801	(+)-10,11-Dihydro-5-methyl-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine
NMDA	*N*-Methyl-D-aspartate
PCP	Phencyclidine
SD	Sprague–Dawley
SR	Serine racemase

Introduction

D-Amino acids are stereoisomers of naturally occurring L-amino acids. They are considered unnatural amino acids and were thought to be absent in higher organisms. However, the development and improvement of analytical methods and instruments have revealed the presence of D-amino acids even in mammalian brains (as reviewed in Konno et al. 2008; Hamase et al. 2009). Here, enzymes that synthesize and degrade D-amino acids have also been discovered, and the significance of these D-amino acids and enzymes is gradually being elucidated (for recent reviews see Pollegioni and Sacchi 2010; Verrall et al. 2010; Nishikawa 2011; Ohide et al. 2011; Wolosker 2011). In this mini-review, we summarize recent findings using mutant mice and rats lacking D-amino-acid oxidase (DAO) activity.

D-Amino acids in the brain

D-Amino acids that have been detected in the mammalian brain include D-serine, D-aspartate, D-alanine, D-leucine, D-proline, and D-glutamate.

D-Serine

A large quantity of D-serine was found in rat brains 20 years ago (Hashimoto et al. 1992) and was later found in human brains (Hashimoto et al. 1993). This D-amino acid is abundant in the cerebral cortex, hippocampus, anterior olfactory nucleus, and amygdala in rats (Schell et al. 1995). This distribution pattern closely resembles that of *N*-methyl-D-aspartate (NMDA) receptors, a subclass of ionotropic glutamate receptors. D-Serine was found to bind to the co-agonist binding site of NMDA receptors and to potentiate neurotransmission (Mothet et al. 2000; see Verrall et al. 2010). NMDA receptors are involved in numerous physiological and pathological processes, including synaptic plasticity, learning, memory, neuronal cell migration, and neural diseases. In fact, D-serine released from glia has been shown to regulate NMDA receptor-dependent long-term potentiation (LTP) and long-term depression (LTD) in hypothalamic and hippocampal slices (Yang et al. 2003; Panatier et al. 2006; Duffy et al. 2008; Benneyworth et al. 2012; Fossat et al. 2012). D-Serine is also present in the cerebellum but only during developmental stages. It is synthesized from L-serine by serine racemase (SR) (Wolosker et al. 1999); in fact, the level of D-serine in the frontal brain of SR knockout mice is decreased to nearly 10 % of the wild-type level (Inoue et al. 2008; Basu et al. 2009; Labrie et al. 2009c; our unpublished data).

D-Aspartate

D-Aspartate was the first D-amino acid found in mammalian brains (Dunlop et al. 1986) and is one of the most abundant D-amino acids in mammals. D-Aspartate is distributed throughout the entire brain; however, the amount of this compound changes during development (see Katane and Homma 2011). In the adult, it is present at high levels in the pineal and pituitary gland and at low levels in the cerebrum, hippocampus, hypothalamus, cerebellum, and medulla oblongata. In the pineal gland, D-aspartate is thought to suppress the synthesis and secretion of melatonin. In the anterior lobe of the pituitary gland, D-aspartate increases the synthesis and secretion of prolactin, luteinizing hormone, and growth hormone. In the intermediate lobe of the pituitary gland, D-aspartate suppresses the level of α-melanocyte stimulating hormone (α-MSH). In the posterior lobe of the pituitary gland and the hypothalamus, D-aspartate modulates the production of oxytocin and vasopressin (see D'Aniello 2007; Katane and Homma 2011, and references therein).

Because D-aspartate is structurally similar to NMDA, it binds to NMDA receptors and potentiates NMDA receptor-mediated neurotransmission (Fagg and Matus 1984; D'Aniello et al. 2011). Therefore, D-aspartate also functions as a neuromodulator in the cerebrum (Huang et al. 2006; Errico et al. 2006). Furthermore, D-aspartate has been shown to be involved in neurogenesis and brain development (Wolosker et al. 2000; Kim et al. 2010).

D-Aspartate is produced from L-aspartate by aspartate racemase (Kim et al. 2010; D'Aniello et al. 2011), but this enzyme needs further characterization. D-Aspartate is degraded by D-aspartate oxidase (see Katane and Homma 2011).

D-Alanine

D-Alanine is present at high concentrations in the pituitary gland and pancreas (Morikawa et al. 2003). Lower levels of D-alanine are present in other regions of the brain. Immunohistochemistry has shown that D-alanine exists in the anterior lobe of the pituitary gland (Etoh et al. 2009). The levels of D-alanine in the pituitary gland as well as in the pancreas and blood fluctuate with the circadian rhythm: the maximum level during the day is approximately four times higher than the minimum level at night (Morikawa et al. 2008). D-Alanine is present in cells containing adrenocorticotropic hormone (ACTH) in the anterior pituitary gland and in the insulin secreting *β*-cells in the islets of Langerhans of the pancreas (Morikawa et al. 2007; Etoh et al. 2009). Therefore, D-alanine is suggested to have some function in the regulation of blood glucose levels through the action of ACTH and insulin (see Hamase et al. 2009). The origin of D-alanine is unclear because no racemase that catalyzes the conversion between L-alanine and D-alanine has been discovered in mammals.

D-Leucine, D-proline and D-glutamate

D-Leucine is present in the brain at very low levels compared with other D-amino acids. The pituitary and pineal glands, as well as the hippocampus, have slightly higher levels of D-leucine than other areas of the brain (Hamase et al. 1997). Similarly, D-proline is also found in the brain at very low concentrations. The amount of D-proline is relatively higher in the pituitary and pineal glands than in other regions of the brain (Hamase et al. 2001).

Low level of D-glutamate has also been detected in the brain (Kera et al. 1995). The functions of these D-amino acids are not known (see Hamase et al. 2005; Verrall et al. 2010).

D-Amino-acid oxidase (DAO)

DAO catalyzes the oxidative deamination of D-amino acids, producing the corresponding 2-oxoacids (α-keto acids), hydrogen peroxide, and ammonia (Krebs 1935). It is a flavoenzyme that uses FAD as a prosthetic group. DAO has wide substrate specificity and oxidizes many neutral and basic amino acids. However, acidic amino acids such as D-aspartate and D-glutamate are not oxidized.

In mammals, DAO is primarily present in the kidney, liver (with the exception of the mouse liver), and brain (Konno et al. 1997). Histochemical staining for DAO activity has indicated that this enzyme exists in glial cells of the cerebellum, pons, and medulla oblongata, whereas it is not present in the forebrain (Horiike et al. 1994; Schell et al. 1995). An enzyme activity assay using HPLC has confirmed this distribution (Hamase et al. 2006a). Unexpectedly, however, DAO mRNA and immunoreactivity against DAO have been detected in the forebrain (Yoshikawa et al. 2004; Verrall et al. 2007; Sacchi et al. 2008; see Verrall et al. 2010). Further investigations are needed to elucidate the function and significance of these mRNA and DAO proteins.

The physiological role of DAO has long been unclear, especially in the brain, as D-amino acids have been considered rare in mammals. A turning point in the field was the discovery of large amounts of D-serine, a known substrate of DAO, in the brains of rodents and humans (Hashimoto et al. 1992, 1993). DAO also attracted much attention when Chumakov et al. (2002) reported that the product of *G72*, a novel gene associated with schizophrenia, interacted with DAO.

Mutant mice lacking DAO activity

To determine the physiological function of DAO, we established a mutant mouse strain (ddY/DAO$^-$) lacking DAO activity (Konno and Yasumura 1983). The ddY/DAO$^-$ mice had a missense mutation (G181R) that causes a complete loss of enzyme activity (Sasaki et al. 1992). These mutant mice, however, did not manifest overt abnormality in appearance. They also did not show any difference in lifespan or reproductive capacity from the control ddY/DAO$^+$ mice. Furthermore, no significant differences were observed in the expression of the Asc-1 transporter (the postulated primary transporter of D-serine), the GlyT1 transporter (a glycine transporter that can also act on D-serine), or SR (Almond et al. 2006). Additionally, the uptake of D-serine into synaptosomes prepared from the cerebellum showed no observable differences. The expression levels of the NR1 subunit of the NMDA receptor showed no change between ddY/DAO$^-$ and ddY/DAO$^+$ mice, which is consistent with the report that DAO knockdown in the cerebellum did not affect the expression of the NR1 or NR2C subunits although reduced the expression of the NR2A subunit (Burnet et al. 2011). These results are parallel to the experimental results in SR knockout mice. Although D-serine levels were dramatically decreased in the forebrain of the knockout mice, significant changes were not detected in the expression of DAO, the subunits of NMDA receptors or D-serine transporters (Labrie et al. 2009c; Horio et al. 2011).

Increase of D-amino acids in the brains of mutant rats and mice lacking DAO activity

A mutant rat strain (LEA/SENDAI) lacking DAO was described recently (Konno et al. 2009; Miyoshi et al. 2011). LEA/SENDAI rats have no DAO activity, DAO protein, or mRNA coding DAO.

The levels of D-serine in the cerebellum, medulla oblongata, and spinal cord were drastically increased in LEA/SENDAI rats compared with Wistar and Sprague-Dawley (SD) rats possessing DAO (Fig. 1a). However, the amounts of D-serine in the olfactory bulb, cerebral cortex, hippocampus, and hypothalamus were not changed among these rats. These patterns were almost the same as those observed in ddY/DAO$^-$ mice (Morikawa et al. 2001; Hamase et al. 2005; Miyoshi et al. 2009). Labrie et al. (2009b) also observed that the D-serine level was not changed in the prefrontal cortex and amygdala in contrast to the slight increase in the hippocampus and the marked increase in the cerebellum of *Dao1*^{G181R} mutant mice, which carry the same mutation as ddY/DAO$^-$ mice but on a C57BL/6J background. These results are consistent with the distribution of DAO activity in the brain: DAO activity is present in the cerebellum and brainstem, but not in the cerebrum (Horiike et al. 1994; Schell et al. 1995).

In contrast to D-serine, the amount of D-alanine was uniformly increased in every area of the brain of LEA/SENDAI rats (Fig. 1b). This increased pattern was the same as that seen in ddY/DAO$^-$ mice (Morikawa et al. 2001; Hamase et al. 2005; Miyoshi et al. 2009). It is probably due to the marked increase in D-alanine in the blood (Morikawa et al. 2007; Miyoshi et al. 2011).

The amount of D-aspartate in all regions of the brain was similar in the ddY/DAO$^-$ and ddY/DAO$^+$ mice (Morikawa et al. 2001; Hamase et al. 2005). This is expected because D-aspartate cannot act as a substrate of DAO.

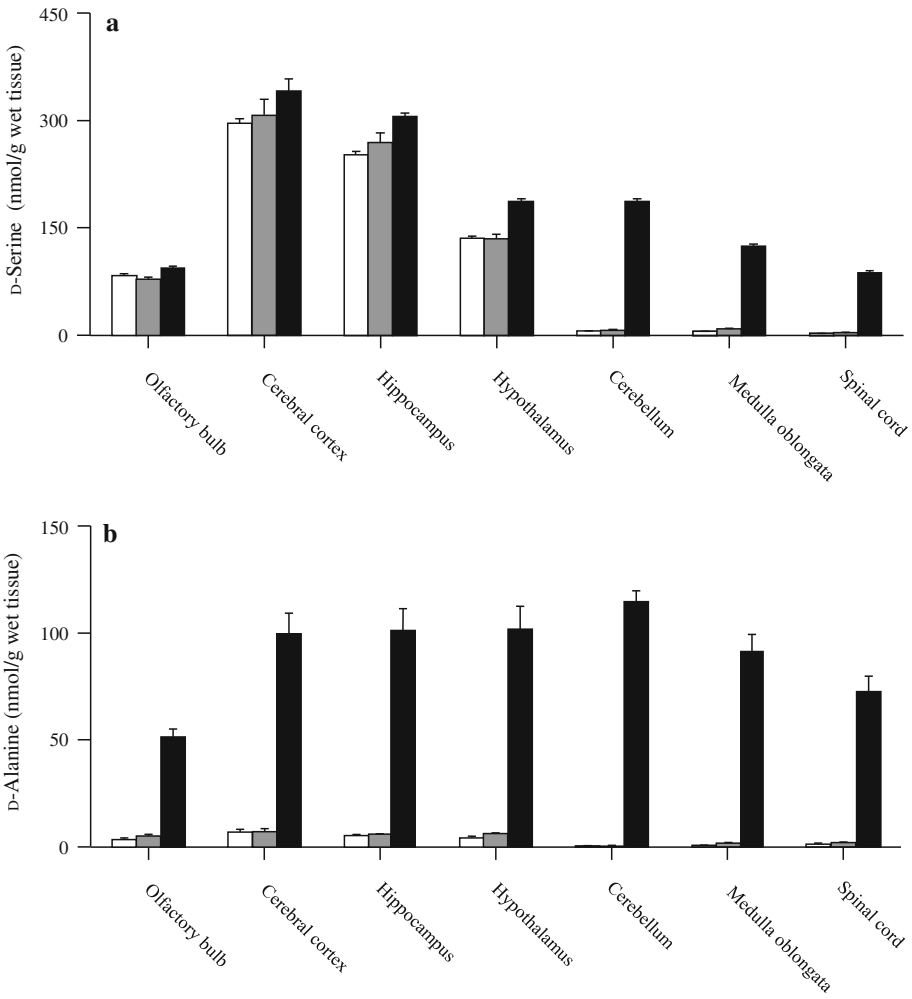

Fig. 1 Amounts of D-serine and D-alanine in the seven regions of the brain of wild-type rats and mutant rats lacking DAO. **a** D-Serine; **b** D-Alanine. *Open bars* Wistar rats possessing DAO. *Gray bars* SD rats possessing DAO. *Closed bars* Mutant LEA/SENDAI rats lacking DAO. Note that the ordinate scale in Fig. 1b is one-third that of Fig. 1a. These figures are reproduced from Miyoshi et al. (2011) with permission from Elsevier

The amount of D-leucine was also increased in every region of the brain of ddY/DAO$^-$ mice (Hamase et al. 2001, 2005). This increased pattern was almost the same as that of D-alanine. This would be also the reflection of the increase of D-leucine in the blood (Hamase et al. 2001).

The amount of D-proline was almost the same between ddY/DAO$^-$ and ddY/DAO$^+$ mice, although slight increases were observed in the pituitary and pineal glands, and cerebellum of ddY/DAO$^-$ mice (Hamase et al. 2001, 2006b). Since the level of D-proline is very low, it is not known whether these tissues have a specific mechanism for the accumulation of D-proline.

The functions of D-serine and DAO in the cerebellum

Large amounts of D-serine are present in the cerebellum of mice and rats during the early postnatal period, but D-serine becomes undetectable 3 weeks after birth (Nagata et al. 1994; Hashimoto et al. 1995). This is because DAO is expressed in the cerebellum after birth, and its activity constantly increases until reaching the adult level after 1 month (Weimar and Neims 1977; Wang and Zhu 2003). The functions of D-serine and DAO have long been unknown, but it has recently been demonstrated that they play essential roles in the development and function of the cerebellum.

In the developing cerebellum, granule cells migrate from the external granular layer to the internal granular layer. In this process, D-serine released from Bergman glia was shown to bind to NMDA receptors on granule cells and to facilitate their migration (Kim et al. 2005). Externally applied DAO or SR inhibitors impeded this migration in cerebellar slices prepared from growing mice, whereas cell migration was restored by the addition of D-serine.

It was also shown that in the immature cerebellum, D-serine released from Bergman glia binds to $\delta 2$ glutamate receptors to regulate LTD at synapses between parallel fibers and Purkinje cells (Kakegawa et al. 2011). This LTD is considered to be the underlying mechanism of learning, memory, and motor coordination (Kashiwabuchi et al. 1995; Kakegawa et al. 2011). This type of LTD is only

observed in cerebellar slices prepared from mice at the developmental stage because D-serine is only found at this stage. However, in ddY/DAO$^-$ mice, LTD induction was observed even in the cerebellar slices prepared from adult mice (Kakegawa et al. 2011). This is because D-serine is present at abundant levels in the cerebellum of these mice even in adulthood.

Collectively, these studies have revealed the significance of D-serine and DAO in cerebellar development and plasticity.

The role of DAO in amyotrophic lateral sclerosis (ALS)

The involvement of D-serine and DAO in neurodegenerative diseases has been clearly demonstrated in ALS. ALS is a progressive neurodegenerative disorder characterized by the selective loss of motor neurons in the spinal cord and brainstem leading to muscle weakness, atrophy, paralysis, and death from respiratory failure (Mitchell et al. 2010; Sasabe et al. 2012). D-Serine levels were found to be elevated in the spinal cord in both human ALS patients and a mouse model of ALS (Sasabe et al. 2007).

Recently, a missense mutation (R199W) in the DAO gene was found in a family with ALS (Mitchell et al. 2010). This mutation markedly reduces DAO activity and promotes the formation of ubiquitinated protein aggregates in the neurons, which led to their degeneration. This effect was also observed when motor neurons were co-cultured with astrocytes expressing transduced R199W DAO. This mutation is rare and was not observed in other ALS patients. However, another heterozygous variant (R38H DAO) was detected in a separate case of familial ALS (Millecamps et al. 2010).

The mutation (G181R) found in ddY/DAO$^-$ mice was transferred by crossing on a C57BL/6 background. The resultant ^{B6}DAO$^{-/-}$ mice developed an abnormal limb reflex (Sasabe et al. 2012). The number and size of motor neurons in the spinal cord were reduced, whereas the amount of D-serine was highly increased. The aged mice manifested muscle atrophy due to axonal degeneration.

In the ALS model mice carrying a missense mutation (G93A) in the superoxide dismutase 1 (*SOD1*) gene, DAO mRNA, DAO protein, and DAO activity were found to gradually decrease in the spinal cord (Sasabe et al. 2012). Consequently, the amount of D-serine increased in the spinal cord concomitantly with the progression of the disease.

These results indicate that DAO keeps the amount of D-serine at a low level in the spinal cord and prevents the overexcitation of motor neurons. However, in the case of ALS, low DAO activity causes the accumulation of D-serine. The excess D-serine overactivates NMDA receptors, causing the excessive influx of calcium ions into neurons. This overexcitation leads to the degeneration of motor neurons. Therefore, DAO seems to play a pivotal role in the pathogenesis of ALS (Sasabe et al. 2012).

Thompson et al. (2012) also found that G93A *SOD1* mice had twofold higher level of D-serine in the spinal cord. They crossed the G93A *SOD1* mice with SR knockout mice to generate double-mutant mice. The level of D-serine in the spinal cord of the double mutants was decreased to half of the level of the G93A *SOD1* mice. Strangely, however, these mice showed earlier symptom onset but survived longer. Curiously again, administration of D-serine to the G93A *SOD1* mice drastically lowered the D-serine level in the spinal cord, though the D-serine level was increased twofold in the brain (Thompson et al. 2012).

Further investigation is needed for the elucidation of etiology of ALS. It is important to clarify the mechanism by which the *SOD1* mutation increases D-serine in the spinal cord. It would be necessary to examine the connection between SOD1 and DAO.

It is conceivable that disorders affecting the levels of D-serine and DAO might also be implicated in some types of cerebrospinal degeneration, which is characterized by the degeneration of motor neurons in the cerebellum, brainstem, and spinal cord similar to ALS.

Pain and DAO

Compared with ddY/DAO$^+$ mice, mutant ddY/DAO$^-$ mice showed an exaggerated response in the second phase of the formalin test (Wake et al. 2001). This experiment indicates that ddY/DAO$^-$ mice suffer from tonic pain more severely. However, the opposite response was observed by Zhao et al. (2008). In addition to the formalin test, ddY/DAO$^-$ mice had longer latent periods before they manifested pain responses in the tail-flick and hot-plate tests. They also showed weaker responses in the acetic acid-induced writhing test (Zhao et al. 2008). Consistently with these results, intravenous injection of a DAO inhibitor into normal mice significantly inhibited pain responses in these tests (Zhao et al. 2008). Therefore, it has been concluded that DAO acts as a pro-nociceptive factor in pain. However, Labrie et al. (2009b) noticed that the latency in the tail-flick test was not different between *Dao1*^{G181R} mutant and wild-type mice.

Zhao et al. (2010) found that tight L5/L6 spinal nerve ligation in rats caused allodynia and increases in DAO mRNA and DAO activity in the lumber spinal cord. Intrathecal injection of sodium benzoate (a classical DAO inhibitor) blocked allodynia in these rats. In addition to sodium benzoate, intrathecal injections of three types of newly developed DAO inhibitors also prevented formalin-induced tonic pain in

a dose-dependent manner. The effectiveness of the inhibitors paralleled the degree of inhibitory activity against rat spinal DAO in vitro (Gong et al. 2011).

Gong et al. (2012) found that DAO inhibitor (CBIO, 5-chloro-benzo[d]isoxazol-3-ol) and morphine showed additive effects on both acute and tonic pain in the formalin test and that CBIO was able to prevent morphine tolerance to analgesia.

Chen et al. (2012) used RNA interference to suppress DAO activity. Intrathecal injections of both siRNA against DAO and recombinant adenovirus that expresses small hairpin RNA against DAO inhibited DAO expression and activity in the spinal cord of rats. These treatments prevented formalin-induced tonic pain, which was consistent with the results of DAO inhibitors.

Lu et al. (2012) found that hydrogen peroxide produced by DAO in the spinal cord is a major culprit of the formalin-induced tonic pain. They suggested that D-serine and NMDA receptors were not directly involved in this process. One unsolved problem in these experiments is the source of the D-amino acid substrates necessary to produce hydrogen peroxide (Lu et al. 2012). SR may be activated after the formalin injection because it has been shown to be induced by inflammatory stimuli (Wu and Barger 2004). LEA/SENDAI rats and ddY/DAO$^-$ mice can be used to determine whether D-amino acids increase in the spinal cord during the second phase of the formalin test.

Maekawa et al. (2012), on the other hand, showed that D-serine was involved in the development of central sensitization of chronic pain. Using the slices of amygdala, they found that the decay time of NMDA receptor-mediated excitatory postsynaptic currents (EPSC) was elongated by arthritis in wild-type mice, but not in SR knockout mice. Application of D-serine augmented EPSC in the arthritic wild-type mice, but not in the arthritic mutant mice. These results suggest that endogenous D-serine influences chronic pain-induced plastic changes in the functions of NMDA receptors.

NMDA receptors and schizophrenia

Schizophrenia is a devastating mental disease affecting nearly 1 % of the population. Although the etiology of schizophrenia is not clear, hypofunction of NMDA receptors is considered to be one of the most plausible causes of this disease (see Nishikawa 2011; Labrie et al. 2012).

A memorable article published 10 years ago indicated that DAO was associated with schizophrenia (Chumakov et al. 2002). This is supposed to be due to the ability of DAO to decrease the level of D-serine, which physiologically binds to and potentiates NMDA receptors: an abnormal increase in DAO activity might result in the hypofunction of the receptors. Since then, a variety of experimental results in humans have been reported to support this hypothesis. (1) Levels of D-serine in cerebrospinal fluid and serum were reduced in schizophrenic patients (Hashimoto et al. 2003; Bendikov et al. 2007). (2) Activity and expression of DAO were augmented in the postmortem brains of patients with schizophrenia (Kapoor et al. 2006; Burnet et al. 2008; Madeira et al. 2008; Habl et al. 2009; Ono et al. 2009). (3) Administration of D-serine with or without antipsychotics improved the symptoms of schizophrenic patients (Tsai et al. 1998; Heresco-Levy et al. 2005; Kantrowitz et al. 2010), and (4) A number of genetic studies have confirmed the association of DAO with schizophrenia (Bendikov et al. 2007; Corvin et al. 2007; Ohnuma et al. 2009; Roussos et al. 2011; see Verrall et al. 2010; Labrie et al. 2012).

However, conflicting results with the aforementioned hypothesis have been put forth. The amount of D-serine in cerebrospinal fluid was not different between schizophrenic patients and normal subjects (Fuchs et al. 2008; Hons et al. 2008). In addition, the genetic association studies could not replicate the association between DAO and schizophrenia (Yamada et al. 2005; Bass et al. 2009; Jönsson et al. 2009; Ohnuma et al. 2010).

This hypothesis was examined using ddY/DAO$^-$ mice. The administration of MK-801 (antagonists of NMDA receptors that induces schizophrenic phenotypes) induced stereotypy in both ddY/DAO$^+$ and ddY/DAO$^-$ mice, but the symptoms were attenuated in the mutant mice (Hashimoto et al. 2005). Methamphetamine caused the similar effect in these mice (Hashimoto et al. 2008). Phencyclidine (PCP, an antagonist of NMDA receptors) induced hyperactivity only in the ddY/DAO$^+$ mice (Almond et al. 2006). L-701,324 (an antagonist of the co-agonist binding site of NMDA receptors) induced ataxia only in the ddY/DAO$^+$ mice. These results may be explained by the enhanced activity of NMDA receptors due to the high levels of D-serine in ddY/DAO- mice antagonizing the effects of MK-801, PCP, and L-701,324. Supporting this assertion, NMDA receptor-mediated neurotransmission was augmented at synapses in the dorsal horn of the spinal cord of ddY/DAO$^-$ mice (Wake et al. 2001).

Behaviors related to NMDA receptor activity have been examined in ddY/DAO$^-$ and *Dao1*^{G181R} mice. In these behavioral tests, the ddY/DAO$^-$ mice and the *Dao1*^{G181R} mice did not always display consistent results. In the prepulse inhibition test, ddY/DAO$^-$ mice showed both an unaltered response (Almond et al. 2006) and an enhanced response (Zhang et al. 2011). In the locomotor activity test, consistent results were not obtained (Hashimoto et al. 2005; Almond et al. 2006; Labrie et al. 2009a; Zhang et al. 2011). In the Morris water maze, ddY/DAO$^-$ mice showed

good spatial memory (Maekawa et al. 2005a), but *Dao1*^{G181R} mice did not (Labrie et al. 2009b). The *Dao1*^{G181R} mice showed improvements in spatial reversal memory and extinction (Labrie et al. 2009a). Female *Dao1*^{G181R} mice exhibited anxiety-like behavior (Labrie et al. 2009a), but ddY/DAO^{-} mice did not (Zhang et al. 2011). These inconsistencies may be ascribed to the differences in the genetic background, experimental environments, and the methods employed.

Inconsistencies in the behavioral tests were also observed in SR knockout mice (see Mori and Inoue 2010; Labrie et al. 2012) and in wild-type mice and rats that were treated with DAO inhibitors (see Verrall et al. 2010; Ferraris and Tsukamoto 2011). Therefore, DAO and D-serine would not be critical factors which cause clear-cut changes in behaviors. Instead, they may be risk factors for schizophrenia. This finding is consistent with the fact that schizophrenia is likely to be caused by the gradual accumulation of small genetic and environmental cues.

Because D-serine potentiates the activity of NMDA receptors and may be effective for the treatment of patients with schizophrenia, DAO inhibitors have been developed at several pharmaceutical companies and tested on mice and rats. The administration of DAO inhibitors clearly increases D-serine in the cerebellum; however, consistent results have not been obtained in the cerebrum since some inhibitors increased cerebral D-serine, whereas others did not (Ferraris et al. 2008). However, some inhibitors were able to ameliorate schizophrenic symptoms in animal models (Adage et al. 2008; see Smith et al. 2010; Ferraris and Tsukamoto 2011). Co-administration of a DAO inhibitor with D-serine increased cerebral D-serine and ameliorated symptoms in animal models more effectively than did the singular treatments (Ferraris et al. 2008; Hashimoto et al. 2009).

A significant and serious problem remains in the relationship between DAO and schizophrenia. This is the mechanism by which DAO affects pathophysiology of schizophrenia. DAO is predominantly present in the cerebellum and brainstem, whereas schizophrenia is an abnormality in the cerebrum. The mechanism that connects this condition with the enzyme is not known. Several possibilities should be considered: (1) There may be cryptic, trace DAO in the vicinity of synapses that cannot be detected with activity staining. Recently, Popiolek et al. (2011) have shown that DAO interacts with synaptic proteins. (2) D-Amino acids other than D-serine, such as D-alanine, may be involved. D-Alanine can serve as a co-agonist of the NMDA receptor (Sakata et al. 1999). The marked increase of D-alanine in the forebrain in the absence of DAO (Fig. 1b) may support this postulate. (3) There may be some mechanism by which DAO in the cerebellum and brainstem affects the function of the cerebrum, and (4) DAO may have other functions in addition to the degradation of D-serine.

Rat DAO

Frattini et al. (2011) have recently shown that recombinant rat DAO differs significantly from human DAO in the enzymatic properties. Due to an extremely low affinity of rat DAO for D-serine, it did not seem to catabolize D-serine efficiently. Indeed, transfection of recombinant rat DAO into glioblastoma cells did not decrease the concentration of D-serine in these cells in contrast to the expression of human homolog. They also reported that there was neither apparent DAO-specific signal in western blot analysis, nor DAO activity in the crude extract of rat brain. Therefore, they have suggested that rat DAO has a different physiological function from human counterpart. However, this hypothesis does not seem consistent with other biological results. First, in LEA/SENDAI rats lacking DAO, D-serine is drastically increased in the cerebellum, medulla oblongata, and spinal cord (Fig. 1a). D-Alanine is also increased in all areas of the brain (Fig. 1b). These patterns are the same as those observed in ddY/DAO- mice (see Hamase et al. 2005), indicating that rat DAO degrades D-serine and D-alanine in the brains of normal Wistar and SD rats. Second, when a DAO inhibitor was administered to rats, the amount of D-serine increased in the cerebellum, but not in the cerebrum (Strick et al. 2011; see Smith et al. 2010), indicating that DAO functions in the cerebellum. Gong et al. (2011) showed that rat spinal DAO was inhibited by several DAO inhibitors in a similar fashion as porcine kidney DAO. Third, histochemically active DAO was present in the rat hindbrain (Horiike et al. 1994; Schell et al. 1995). Fourth, many researchers have actually demonstrated DAO activity in the homogenates of rat brain and spinal cord (Gaunt and de Duve 1976; Weimar and Neims 1977; Kappor and Kapoor 1997; Zhao et al. 2010). These results suggest that rat DAO is functioning in the central nervous system (CNS).

Prospects

The functions of D-amino acids and DAO in the CNS have been considerably delineated in recent years. Mutant mice and rats lacking DAO activity have contributed to the determination of their roles. Further investigations using these animals will lead to an even more complete understanding of the functions of D-amino acids and DAO in physiology and pathology. One large problem at present is the lack of an explanation of the molecular mechanism that connects DAO to schizophrenia, if any. Other mutant mice

or rats possessing an increased level of DAO activity would be useful in addressing this problem. Conditional mutants in DAO may also be more helpful.

For the treatment of schizophrenia, large amounts of D-serine were administered to schizophrenic patients (Tsai et al. 1998; Kantrowitz et al. 2010). However, D-serine is nephrotoxic to rats (Carone and Ganote 1975). Using LEA/SENDAI rats, Maekawa et al. (2005b) have clearly shown that DAO is responsible for this nephrotoxicity. Because nephrotoxicity of D-serine was not observed in mice, hamsters, gerbils, guinea pigs, rabbits, or dogs (Morehead et al. 1946), it is not clear whether humans are subject to D-serine nephrotoxicity. In any case, it would be advantageous to administer D-serine together with DAO inhibitor(s) to avoid possible kidney damage and the breakdown of D-serine by DAO. Another concern is a risk of overstimulation of NMDA receptors with high level of D-serine, leading to neurodegeneration as observed in ALS. Therefore, careful consideration and follow-up would be necessary for the administration of D-serine.

Conflict of interest The authors declare that they have no conflict of interest.

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