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Biochemistry of D-aspartate in mammalian cells

Review Article

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Summary. Recent investigations have shown that D-aspartate (D-Asp) plays an important physiological role(s) in the mammalian body. Here, several recent studies of free D-Asp metabolism in mammals, focusing on cellular localization in tissues, intracellular localization, biosynthesis, efflux, uptake and degradation are reviewed.

D-Asp in mammalian tissues is present in specific cells, indicating the existence of specific molecular components that regulate D-Asp levels and localization in tissues. In the rat pheochromocytoma cell line (PC12) and its subclones, D-Asp is synthesized intracellularly, most likely by Asp racemase(s). Endogenous D-Asp apparently has two different intracellular localization patterns: cytoplasmic and vesicular. In PC12 cells, D-Asp release can occur through three distinct pathways: 1) spontaneous, continuous release of cytoplasmic D-Asp, which is not associated with a specific stimulus; 2) release of cytoplasmic D-Asp via a volume-sensitive organic anion channel that connects the cytoplasm and extracellular space; 3) exocytotic discharge of vesicular D-Asp. Under certain conditions, D-Asp can be released via a mechanism that involves the L-Glu transporter. D-Asp is thus apparently in dynamic flux at the cellular level to carry out its physiological function(s) in mammals.

Keywords: D-Aspartate – Localization – Biosynthesis – Efflux – Uptake – Degradation

1. Introduction

Since its discovery in invertebrates (D'Aniello and Giuditta, 1977), free D-aspartate (D-Asp) has been identified in a variety of organisms, including microorganisms (Long et al., 2001b; Matsumoto et al., 1999; Nagata et al., 1999c), plants (Brückner and Westhauser, 2003), and lower animals (Assisi et al., 2001; Felbeck, 1985; Okuma et al., 1995; Raucci et al., 2005; Watanabe et al., 1998), mammals and humans (Dunlop et al., 1986; Hashimoto et al., 1993a, b, 1995; Man et al., 1983, 1987). Many studies of lower animals and mammals have examined the tissue distribution of D-Asp (D'Aniello and Giuditta, 1978; Hashimoto and Oka, 1997; Imai et al., 1996; Okuma et al., 1998; Shibata et al., 2001), developmental regulation of D-Asp levels in organs (D'Aniello et al., 2005b; Hamase et al., 1997; Hashimoto et al., 1995), and localization of D-Asp within different tissues (Homma, 2002; Schell et al., 1997). In addition, the biological and physiological activities of D-Asp in animals and humans (Boehning and Snyder, 2003; Di Fiore et al., 1998; Furuchi and Homma, 2005; Homma, 2002; Raucci et al., 2005), and the pathological significance of D-Asp in humans (D'Aniello et al., 1998a, 2005a; Fisher et al., 1994) have also been examined. The evidence to date suggests that D-Asp plays an important physiological role(s) in living organisms, but many details remain unclear. In this short communication from the viewpoint of cell biology, I will briefly review our current understanding of D-Asp metabolism and regulation, including cellular localization within tissues, intracellular localization, biosynthesis, cellular uptake and efflux, and metabolic degradation in mammalian cells. The biological and physiological activities of D-Asp in mammals have been the subject of previous review articles (Furuchi and Homma, 2005; Homma, 2002).

2. Cellular localization in tissues

The location of D-Asp within mammalian tissues has been examined using a stereo-specific anti-D-Asp antibody (D'Aniello et al., 1996; Homma, 2002; Masuda et al., 2003; Schell et al., 1997; Wang et al., 2000; Wolosker et al., 2000). In the rat adrenal medulla, D-Asp is closely

associated with adrenaline-cells (A-cells), which account for approximately 80% of the total number of chromaffin cells in the tissue, and which make and store adrenaline. D-Asp appears to be absent from noradrenaline-cells (NA-cells), which comprise approximately 20% of the total number of chromafin cells in the adrenal medulla, and which make and store noradrenaline. D-Asp oxidase, which catalyzes oxidative deamination of D-Asp, appears to be present only in NA-cells, suggesting that the lack of D-Asp in these cells is due to D-Asp oxidase-mediated metabolism of D-Asp (Schell et al., 1997). In the rat adrenal cortex, the distribution of D-Asp changes during development. At 3 weeks of age, D-Asp immunoreactivity is prominent in the cytoplasm of cells in the inner zones, zona fasciculate (ZF) and zona reticularis (ZR). It is absent from cells in the outermost zone, zona glomerulosa (ZG). At 8 weeks of age, D-Asp localization is apparently reversed: immunoreactivity in the ZF and ZR decreases, and that in the ZG becomes apparent (Sakai et al., 1997). The innermost regions (ZF and ZR) synthesize and secrete glucocorticoids, whereas the outermost zone ZG secretes mineralocorticoids. Furthermore the maturation periods of these two regions are different. It has been suggested that developmental changes in the localization of D-Asp reflects the participation of D-Asp in the development and maturation of steroidogenesis in rat adrenal cortical cells (Sakai et al., 1997). Previous reports have indicated that D-Asp is involved in steroid hormone synthesis and secretion in mammals as well (D'Aniello et al., 1996; Nagata et al., 1999a, b).

In the anterior lobe of the rat pituitary gland, five different hormones are produced and secreted by distinct cell types: growth hormone (GH), secreted by somatotrophs; prolactin (PRL), secreted by mammotrophs; adrenocorticotropic hormone (ACTH), secreted by corticotrophs; thyroid stimulating hormone (TSH), secreted by thyrotrophs; and gonadotropic hormone (GTH), secreted by gonadotrophs. Of these five cell types, D-Asp immunoreactivity is observed specifically in PRL-producing mammotrophs, or in a closely related type of cell (Lee et al., 1999). In fact, D-Asp is believed to stimulate PRL secretion (Long et al., 2000; D'Aniello et al., 2000b; Pampillo et al., 2002). In the posterior lobe of the gland, intense immunoreactivity of D-Asp is observed in the nerve processes and terminals of neurons (magnocellular neurons) that originate in the supraoptic and paraventricular nuclei of the hypothalamus (Schell et al., 1997; Wang et al., 2000). D-Asp immunoreactivity in the magnocellular neurons of both nuclei has been observed (Schell et al., 1997), and D-Asp has been shown to modulate oxytocin and/or vasopressin production, which take places in the neurons (Pampillo et al., 2001; Wang et al., 2000). D-Asp also induces expression of the relevant gene (oxytocin gene) (Wang et al., 2000), and is present in heterochromatin in the nucleoli of the magnocellular neurons, suggesting that it has a role in gene expression (Wang et al., 2002).

In the rat brain, D-Asp immunoreactivity is observed in distinct nerve cell populations, and its localization is almost the inverse of D-Asp oxidase, suggesting that D-Asp oxidase destroys endogenous D-Asp, similar to what is observed in the rat adrenal medulla (see above) (Schell et al., 1997). D-Asp levels in the brain are highest in embryonic stages, and decline during postnatal development. In the rat embryonic brain, D-Asp immunoreactivity is mainly observed around the hindbrain, and then spreads into the forebrain. It first appears in the cell body of neurons in the outer layer of the neural epithelium. It is subsequently observed in the processes of neuroblasts, and is present exclusively in axons, once a distinct axonal layer is established (Sakai et al., 1998b). Just after parturition (P0), D-Asp is present in the cortical plate and subventricular zone in the cerebral cortex, implying a close association of D-Asp with the region that regulates neural development (Wolosker et al., 2000). During postnatal development, immunoreactivity in the brain decreases, and in adult rats D-Asp is almost undetectable, except in limited regions, such as the hypothalamic nuclei described above (Wolosker et al., 2000).

D-Asp localization has also been examined in rat testis (D'Aniello et al., 1996; Sakai et al., 1998a), rat retina (Lee et al., 1999), rat pineal gland (Lee et al., 1997), rat salivary gland (Masuda et al., 2003) and human spermatozoa (D'Aniello et al., 2005a).

3. Intracellular localization

As described in the section above, D-Asp immunoreactivity is present in the nucleoli of the magnocellular neurons of the rat hypothalamic supraoptic nucleus. Immunoreactivity is predominantly associated with heterochromatin, and is undetectable in other subcellular structures, including the nucleoplasm and cytoplasm (Wang et al., 2002). D-Asp has not been found in astrocytes of the supraoptic nucleus, or in other cells outside the nucleus. In microglial cells of the posterior lobe of the pituitary, a similar localization pattern of D-Asp in heterochromatin is observed. This localization pattern suggests that D-Asp is involved in the control of gene expression. The mechanism could involve D-Asp directly interacting with DNA, or with

nuclear protein (s) involved in the regulation of gene transcription (Wang et al., 2002).

Subcellular localization of D-Asp in cultured mammalian cells such as PC12 cells, PC12 subclones, and GH_3 cells, a rat pituitary tumor cell line, has been reported (Koyama et al., 2006; Long et al., 1998, 2000, 2001a; Nakatsuka et al., 2001). As described in more detail below, several studies have demonstrated that in PC12 cells (including a PC12 subclone) and GH_3 cells, D-Asp is synthesized intracellularly (Long et al., 1998, 2000, 2002). Immunoreactivity of D-Asp is predominantly cytoplasmic in PC12 and GH_3 cells, based on light microscopy, but the relative intensity of staining in a population of cells differs. Thus individual cells have different levels of D-Asp immunoreactivity. (Long et al., 1998, 2000). This difference is more pronounced in 2068 cells, a subclone of PC12 cells. It appears that D-Asp levels in 2068 cells change during the cell cycle, although additional experiments are needed to further clarify these changes (Long et al., 2001a).

In sub-cellular fractionation experiments using PC12 cells, D-Asp is recovered predominantly in the cytosolic fraction following homogenization of the cells. A scarce amount is recovered in the vesicular fraction. In contrast, catecholamine (dopamine) is present in the vesicular fraction (large dense-core vesicles, LDCV) (Koyama et al., 2006). Immunocytochemical staining with anti-D-Asp antibody followed by confocal laser scanning fluorescence microscopy revealed that D-Asp is distributed homogeneously throughout the cytoplasm surrounding the cell nucleus. This observation is consistent with the sub-cellular fractionation results described above.

On the contrary, a different intracellular distribution of D-Asp has been reported (Long et al., 2001a; Nakatsuka et al., 2001). D-Asp immunoreactivity was associated with particulate structures that co-localized with dopamine and a marker protein for LDCV (chromogranin A). D-Asp was also recovered in the vesicle fraction following subcellular fractionation. These results were observed in PC12 cells (Nakatsuka et al., 2001). We reported similar results that D-Asp was associated with granule-like structures bearing fluorescent grains that were present around the nuclei of the cells (Long et al., 2001a). These results were obtained with 2068 cells, a spontaneously occurring PC12 flat cell variant (Ramachandran et al., 1993; Zheng et al., 1996). In light of these apparent discrepancies, it is possible that D-Asp has two different intracellular localization patterns, cytoplasmic and vesicular. The PC12 cells used by Nakatsuka et al. (2001) are most likely a spontaneously occurring flat cell variant of PC12 cells, similar to 2068 cells. In addition, there is likely to be some yet undefined molecular component(s) in 2068 cells, which function to localize D-Asp in vesicles. As described below (Section 5), endogenous D-Asp in PC12 cells is released from these distinct storage sites through distinct pathways depending on the physiological and pathological state of the cell.

4. Biosynthesis

Through the use of several different methods, including HPLC analysis, immunocytochemical staining with anti-D-Asp antibody, and reactivity of D-Asp-specific oxidase, it has been shown that PC12 cells contain D-Asp, and that D-Asp levels, both intracellular and in the cell culture medium, increase over time (Long et al., 1998). Since extracellular D-Asp is not taken up by cells, and the cells do not express the L-Glu transporter, intracellular biosynthesis of D-Asp is most likely occurring in these cells (Long et al., 1998). D-Asp synthesis has also been observed in a subclone of PC12 cells (Long et al., 2002), and in GH_3 cells (Long et al., 2000). In primary cultures of rat embryonic neurons, high levels of endogenous D-Asp were detected, and [^{14}C]-D-Asp biosynthesis was demonstrated by using [^{14}C]-L-Asp as precursor molecule (Wolosker et al., 2000). Treatment of these neuronal cultures with amino-oxyacetic acid (AOAA) markedly inhibited the accumulation of [^{14}C]-D-Asp (Wolosker et al., 2000). Since AOAA is a potent inhibitor of pyridoxal phosphate (PLP)-dependent enzymes, this result suggests that a PLP-dependent enzyme is involved in the conversion of L-Asp to D-Asp. A likely candidate is a racemase, and in fact, an Asp-specific, PLP-dependent racemase of animal origin, the bivalve, has been cloned and characterized (Abe et al., 2005; Shibata et al., 2003a, b; Watanabe et al., 1998). An Asp-specific racemase-like activity, which converted L-Asp to D-Asp, has also been reported in other animals, including the cephalopod (D'Aniello et al., 2005b), frog (Raucci et al., 2005), lizard (Assisi et al., 2001) and mollusk (Spinelli et al., 2005). Interestingly, serine racemases of mammalian origin have also been cloned (Wolosker et al., 1999), and are presumed to be involved in the synthesis of D-serine, a neuromodulator of the NMDA receptor.

An alternative pathway of D-Asp synthesis involves a PLP-dependent D-amino acid aminotransferase, which can transfer an amino group from another D-amino acid to oxaloacetic acid, producing D-Asp. D-Asp may also be produced by degradation of proteins that contain D-Asp residues, and the subsequent release of free D-Asp

(Kinouchi et al., 2004). For example, it is known that L-asparaginyl and L-aspartyl residues in proteins undergo spontaneous isomerization reactions under physiological conditions, which can generate isoaspartyl and D-Asp residues (Clarke, 2003; Fujii, 2002; Reissner and Aswad, 2003).

In mammalian tissues, the synthesis of a novel D-amino acid, *N*-methyl-D-aspartate (NMDA), from D-Asp has been reported (D'Aniello et al., 2000a, b). Synthesis is presumably catalyzed by a methyltransferase that uses *S*-adenosyl-L-methionine (SAM) as methyl donor and D-Asp as methyl acceptor. Significant levels of NMDA synthesis have been observed in the rat hypothalamus, hippocampus and anterior pituitary gland. Endogenous NMDA is normally present in mammalian tissues (D'Aniello et al., 2000a), and has been detected in lower animals as well, including bivalves and snails (Shibata et al., 2001).

5. Efflux

D-Asp efflux in vivo appears to occur in tissues that contain significant amounts of D-Asp. In the adult rat testis, significant levels of D-Asp have been detected, comprising approximately 40% of the total amount of Asp (Sakai et al., 1998a). D-Asp is present in the elongate spermatids within the seminiferous tubules (Sakai et al., 1998a). The concentration of D-Asp in testicular venous blood plasma is much higher than in peripheral blood plasma (D'Aniello et al., 1998b), suggesting that it is released from the testis in vivo.

D-Asp release from rat adrenal tissue slices has been reported (Wolosker et al., 2000). Depolarization with a high concentration of KCl, or treatment with acetylcholine (ACh), resulted in the release of a significant amount of endogenous D-Asp from the tissue. L-Asp was also released, but the magnitude of release was lower. D-Asp release was Ca^{2+}-dependent, and was inhibited by extracellular EGTA. Interestingly, EGTA also significantly suppressed the basal release in the absence of stimulation (Wolosker et al., 2000).

In the rat pineal gland, a markedly high level of D-Asp is present within the pinealocytes, the parenchymal cells of the tissue (Lee et al., 1997). In primary cultures of rat pinealocytes however, cellular levels of D-Asp were marginal, most likely because of leakage during the process of isolation and culture. When these cells were pre-loaded with D-Asp, a significant level of D-Asp release was observed upon stimulation with noradrenaline (Takigawa et al., 1998).

These studies clearly demonstrated that D-Asp can be released into the extracellular milieu, but the specific pathway(s) of release remain to be elucidated. Detailed characterization of the pathway(s) will certainly aid our understanding of the physiological significance of D-Asp in mammals. We therefore have been studying the efflux of D-Asp in cultured mammalian cells. Our results to date suggest that three distinct release pathways are functioning in PC12 cells and PC12 subclones. In our studies, we used three cell lines: PC12, MPT1 and 2068 cells. MPT1 and 2068 cells are spontaneously occurring flat cell variants of PC12 cells (Ramachandran et al., 1993; Zheng et al., 1996). It is important to note that MPT1 cells express the L-Glu transporter (a subtype of GLAST), and can actively take up extracellular D-Asp (Adachi et al., 2004; Ramachandran et al., 1993; Zheng et al., 1996). In contrast, PC12 cells do not express the transporter, and can not take up D-Asp from the extracellular environment (Long et al., 1998; Ramachandran et al., 1993; Zheng et al., 1996).

As described earlier, in PC12 cells, D-Asp is synthesized intracellularly, and D-Asp levels inside the cells and in the cell medium increase over the duration of the culture (Adachi et al., 2004; Long et al., 1998). This result suggests that D-Asp is synthesized by the cells, and is spontaneously and continuously released into the medium, with no specific stimulation (Fig. 1A). Since PC12 cells do not express the L-Glu transporter, this release involves a mechanism that is independent of the transporter (e.g. it does not occur by L-Glu transporter-mediated reverse transport, see below). Endogenous D-Asp is predominantly located in the cytoplasm, since it is recovered in the cytosolic fraction following homogenization and

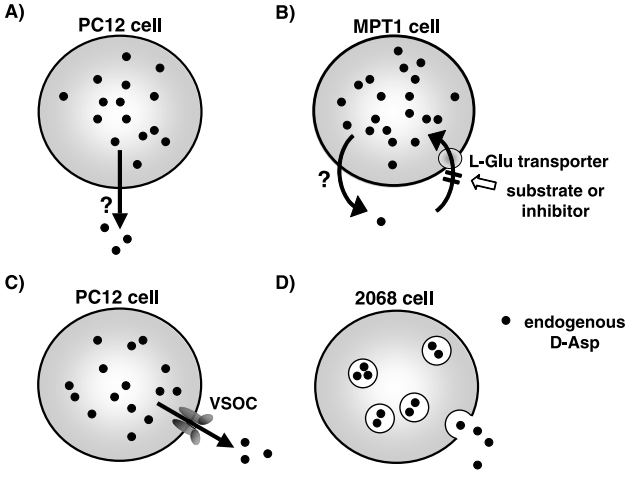

Fig. 1. Release pathways of endogenous D-Asp operating in PC12 cells and its subclones

subcellular fractionation (Koyama et al., 2006). In addition, immunocytochemical staining with anti-D-Asp antibody indicates that D-Asp is distributed homogeneously throughout the cytoplasm (Koyama et al., 2006).

The spontaneous, continuous release pathway was compared to the exocytotic release of dopamine in PC12 cells. It is well documented that dopamine is present in LDCVs and that it is released into the extracellular space through an exocytotic pathway. The kinetic profile of spontaneous, continuous release of D-Asp was distinct from the exocytotic release of dopamine. Furthermore, D-Asp release was insensitive to a voltage-gated Ca^{2+} channel blocker, and to the silencing of the SNARE protein, SNAP-25, whereas dopamine release was significantly inhibited under these conditions (Koyama et al., 2006). Taken together, these results indicate that a spontaneous, continuous release of endogenous D-Asp occurs through efflux of cytoplasmic D-Asp, and that the vesicular exocytotic pathway is not substantially involved (Fig. 1A). However, the detailed mechanism and molecular nature of the pathway remain to be defined.

Our investigations of the transmembrane efflux of D-Asp in PC12 cells revealed a second efflux pathway, via the volume-sensitive organic anion channel (VSOC), which connects the cytoplasm and extracellular space (Fig. 1C). When PC12 cells were exposed to hypotonic medium, significant amounts of endogenous D-Asp were released through VSOC, which opens in response to hypotonic stimulation (Koyama et al., 2006). Putative blockers of VSOC substantially inhibited the observed efflux of D-Asp. It is noteworthy that VSOC blockers were unable to inhibit the spontaneous, continuous release of D-Asp, suggesting that this release pathway is distinct from efflux via VSOC. Interestingly, stimulating the cells with agonists (e.g. ACh stimulation), increased the level of hyposmolarity-induced D-Asp efflux (Koyama et al., 2006).

Similarly to PC12 cells, D-Asp is also synthesized in MPT1 cells (Long et al., 2002). Unlike PC12 cells however, MPT1 cells express the L-Glu transporter. Interestingly, addition of L-Glu to MPT1 culture medium resulted in the accumulation of D-Asp in the medium (Adachi et al., 2004). It has now been concluded, through various lines of evidence, that there is a dynamic exchange of D-Asp between the intra- and extracellular spaces during the culture of MPT1 cells (Adachi et al., 2004; Koyama et al., 2005). As described above, D-Asp is spontaneously and continuously released into the extracellular space. It is also actively and continuously taken up into the cell via an L-Glu transporter-mediated pathway (Adachi et al., 2004; Koyama et al., 2005) (Fig. 1B). During active L-Glu-mediated transport, a low level of extracellular D-Asp is maintained (Long et al., 2002); when substrates (or inhibitors) of the transporter (e.g. L-Glu) are present, they competitively inhibit D-Asp-uptake, resulting in the accumulation of D-Asp in the medium (Fig. 1B).

Immunocytochemical staining of D-Asp in 2068 cells revealed a different pattern of intracellular distribution from that in PC12 cells (Long et al., 2001a). As described in the section 3, D-Asp is found associated with granule-like structures around the nuclei in 2068 cells. Since 2068 cells express the L-Glu transporter, D-Asp can be taken up into these cells. When the cells were preloaded with [^{14}C]-D-Asp, [^{14}C]-D-Asp efflux was Ca^{2+}-dependent, and was stimulated by depolarization with high concentration of KCl or a Ca^{2+} ionophore (Long et al., 2001a). These results suggest that D-Asp is released from 2068 cells via an exocytotic pathway, which is markedly different than what was observed in PC12 cells. An exocytotic release pathway for D-Asp was also reported by Nakatsuka et al. (2001). In this study, D-Asp immunoreactivity was associated with particulate structures, and D-Asp co-localized with dopamine in LDCV. D-Asp was also released by high concentrations of KCl, or by a neurotoxin, in a manner similar to dopamine. These studies were done in PC12 cells, and contradict our results obtained with PC12 cells. One possible explanation for the differences is that the PC12 cells employed by Nakatsuka et al. (2001) were a spontaneously occurring flat variant of PC12 cells similar to 2068 cells. Presumably, there are molecular component(s) (such as vesicular transporters) expressed in these cells that are involved in the accumulation of D-Asp in intracellular vesicles, allowing for exocytotic release.

In summary, there appear to be three distinct release pathways of D-Asp in PC12 cells and its subclones: (1) a spontaneous, continuous release pathway of cytoplasmic D-Asp (Fig. 1A); (2) release of cytoplasmic D-Asp via a VSOC-dependent pathway (Fig. 1C); and (3) exocytotic discharge of vesicular D-Asp (Fig. 1D). Conceivably, these pathways are selectively employed depending on the functional state of the cells in vivo. Additional studies on the mechanism of D-Asp efflux via these different pathways are certainly warranted.

6. Uptake

The transporter that is responsible for the uptake of extracelluar D-Asp into cells is most likely the L-Glu transporter (as described in the previous sections). In addition to L-Glu and L-Asp, this transporter has an

affinity for D-Asp, and in all the subtypes characterized to date, the affinity for D-Asp is almost similar to that of L-Glu or L-Asp (Arriza et al., 1994, 1997; Palacín et al., 1998). Interestingly, the affinity of the L-Glu transporter for D-Glu is so low that it is essentially not transported.

It has been proposed that the L-Glu transporter catalyzes the release of intracellular L-Glu via reverse transport, or via heteroexchange with extracellular substrates (Attwell et al., 1993; Levi and Raiteri, 1993; Nicholls and Attwell, 1990). In a similar manner, the transporter could be involved in the release of endogenous D-Asp. In cultures of mouse neurons or astrocytes radiolabeled D-Asp that was preloaded into the cells was released in the presence of transporter substrates, suggesting that release occurred via a heteroexchange mechanism (Anderson et al., 2001; Bak et al., 2003). Under conditions of energy deprivation that mimic ischemia in vivo, the efflux of radiolabeled D-Asp increased, and was sensitive to transporter inhibitors (Anderson et al., 2001). These results demonstrate that exogenously added D-Asp can be released from cells via an L-Glu transporter-dependent pathway, but additional studies are necessary to determine whether endogenous D-Asp is also released via this mechanism. In MPT1 cells that are cultured under normal conditions, the efflux of endogenous D-Asp via a heteroexchange mechanism is probably marginal, since a non-transportable inhibitor, TFB-TBOA, led to a higher level of efflux of D-Asp than a transportable inhibitor, DL-TBOA (Koyama et al., 2005). Under normal conditions, endogenous D-Asp is apparently in dynamic flux, whereby endogenous D-Asp is spontaneously released by an undefined pathway(s), and continuously and actively taken up by the transporter (Fig. 1B).

As described above, under certain conditions, D-Asp may be released by a mechanism that involves the L-Glu transporter. In the rat, however several types of cells can be identified in which the L-Glu transporter is essentially not expressed, and endogenous D-Asp is detected (Lee et al., 2001). In these cells, D-Asp is probably released by a mechanism that is independent of the transporter as depicted in Fig. 1. Thus, the release pathway of D-Asp can vary with cell type, and functional state of the cell in vivo.

7. Degradation

In mammalian tissues, two types of degradative enzymes are found that are stereospecific to D-amino acids: D-amino acid oxidase, and D-Asp oxidase. The latter acts on acidic D-amino acids such as D-Asp, D-Glu and NMDA, while the former acts on neutral as well as basic D-amino acids (D'Aniello et al., 1993). D-Asp oxidase is an FAD-containing enzyme that catalyzes the oxidative deamination of the acidic D-amino acids to generate the corresponding α-keto acids, together with hydrogen peroxide and ammonium ions. This enzyme is located in the peroxisome, where hydrogen peroxide, a compound that is toxic to cells, can be detoxified by catalase (Van Veldhoven et al., 1991). cDNA clones of D-Asp oxidase have been isolated from bovine kidney and human brain (Setoyama and Miura, 1997; Simonic et al., 1997).

Administration of D-Asp to rodents has been reported to increase D-Asp oxidase activity in the liver and kidney (Nagasaki, 1994; Yamada et al., 1989). This result suggests that the enzyme is induced by D-Asp. In fact, D-Asp oxidase activity in yeast (*Cryptococcus humicolus*) was significantly increased when the yeast were cultured in medium containing D-Asp as a nitrogen source (Yamada et al., 1996). D-Asp oxidase mRNA levels also increased, indicating that transcription of the gene is regulated by D-Asp. Interestingly the induction was specific to D-Asp, whereas L-Asp and D-Glu were ineffective (Takahashi et al., 2004). D-Asp oxidase expression in mammals is presumed to be regulated in a similar way; however, the details remain to be clarified.

In yeast, D-Asp oxidase plays an essential role in the assimilation and detoxification of D-Asp (Takahashi et al., 2005). Although the physiological role of the enzyme in mammals has yet to be defined, it presumably functions in certain tissues to regulate D-Asp levels (Schell et al., 1997) (also see Section 2). A recent study indicated that a human gene located on chromosome 13q34 and genetically linked to schizophrenia (G72) encodes a protein that interacts with and activates D-amino acid oxidase (Chumakov et al., 2002). In the brain, D-amino acid oxidase regulates the amount of D-serine, which is an allosteric activator of the NMDA receptor. If the protein encoded by G72 was overproduced, NMDA receptor activity may be reduced due to the decrease in D-serine levels. It has recently been proposed that hypofunctioning of the NMDA receptor signaling pathway could lead to a predisposition to schizophrenia (Nishikawa, 2005; Snyder and Kim, 2000). Several candidate genes for schizophrenia have been reported (Owen et al., 2005), including G72 (now designated as D-amino-acid oxidase activator, DAOA) and D-amino acid oxidase itself. Data on the pathological roles of D-Asp oxidase are not available at present, and further studies are anticipated.

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