D-ASPARTATE IN THE MAMMALIAN BODY

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(Abstract)

After some doubt, it has now been confirmed that D-amino acids occur naturally in mammalian tissues. In this review, I describe our work with D-aspartate. Immunohistochemical staining reveals that D-aspartate is found in specific cells at distinct periods during the development of rat brain, adrenal, pineal, and pituitary glands, and testis. D-Aspartate appears to be synthesized by the pituitary gland and testis and then secreted into the vascular system which transports it to tissues such as the adrenal and pineal glands, which take it up using the L-Glu transporter. The D-aspartate synthesized by the anterior lobe of the pituitary gland may stimulate prolactin-producing cells to secrete more prolactin in an autocrine and paracrine fashion. In the testis, D-aspartate produced inside the seminiferous tubules may act in a paracrine fashion on Leydig cells that reside outside the tubules to increase their testosterone production by stimulating their expression of Steroidogenic Acute Regulatory protein gene. D-Aspartate in the pineal gland, apparently primarily derived from outside the tissue, suppresses the melatonin secretion by the parenchymal cells (pinealocytes). Studies with cultured mammalian cell lines reveal that intracellular D-aspartate concentrations appears to change during the cell cycle and can be regulated by the cell density of the culture. These studies also showed that mammalian cells contain all the molecular components needed to regulate D-aspartate homeostasis, as they can biosynthesize, release, take up, and degrade D-aspartate. D-Aspartate thus appears to function in the mammalian body as a novel type of a messenger.

(Key Words) D-aspartate, enantioseparation, immunohistochemical localization, melatonin, testosterone, steroidogenic acute regulatory protein, elongate spermatid, prolactin, cell density

Various important amino acid functions appear to be performed only by the L-forms of amino acids, not the D-forms, and D-amino acids have consequently been regarded as unnatural isomers or laboratory artifacts. However, recent reports have demonstrated that a variety of D-amino acids do occur in mammalian tissues in their free form or as protein components. The physiological roles D-amino acids play in mammals are thus currently being investigated. We have been studying D-aspartate (D-Asp) and review here our observations on where and when during the development of the mammalian body this isomer is found. We also describe the evidence that reveals the mechanisms that influence D-Asp levels in cells or within tissues and the biological activities of this isomer.

1. Quantification of D-Asp in different mammalian tissues during development

Enantioseparation and detection of D-Asp in the complex biological samples derived from mammalian tissues is difficult because of the large amounts of L-Asp and other

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biomatrices present in these tissues. To overcome these difficulties, we have established and now routinely employ a highly sensitive method with which we can quantify the D-Asp in mammalian tissues [1]. This method involves the fluorogenic derivatization of amino acids using 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) (Fig. 1a), after which the Asp fraction is isolated by reverse-phase HPLC employing an octadecylsilica column. This is followed by the enantioseparation of D-Asp from L-Asp by using the Pirkle-type chiral columns OA2500 or OA3100 (Fig.1 b). To reduce the assay time, we have recently also developed a new HPLC system in which the two chromatographic steps are linked by an automated column-switching system [2].

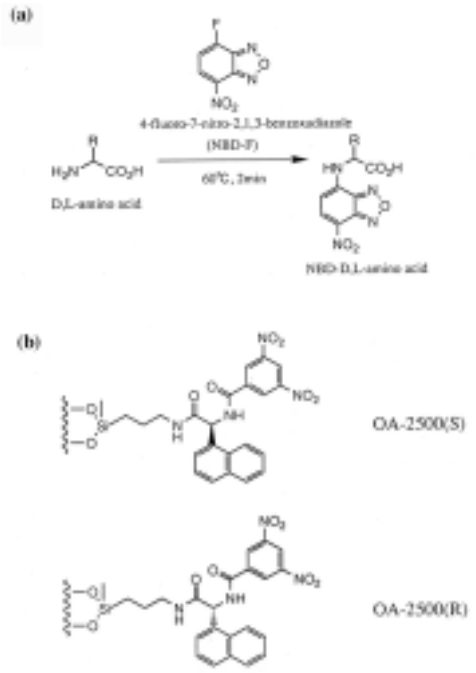

Fig. 1 (a) Fluorogenic derivatization of an amino acid with NBD-F
(b) Structures of Pirkle-type chiral columns, Sumichiral OA-2500 (S) and OA-2500 (R).

We have used this method to quantitate the D-Asp levels and their changes during development in a variety of rat tissues, including the brain, the pineal, pituitary, and adrenal glands, and the testis [1, 3-5]. The D-Asp levels in each tissue follow unique development-related patterns. Regarding the brain, various reports including our own have shown that high concentrations of D-Asp are present in the cerebral hemisphere of rat and chick embryos and that these levels decline rapidly after delivery [1, 6, 7]. This pattern also occurs in humans as at week 14 of human gestation, as much as 60% of the total Asp in the prefrontal cortex is in the D-form [8]. The D-form levels then rapidly decrease to trace levels at birth and remain low thereafter. In contrast, D-Asp concentrations in the rat pineal gland and testis increase as development proceeds and in adult rats the D% (proportion of D-Asp in the total Asp) is as high as 30-40%. In the rat adrenal gland, D-Asp levels transiently increase at 3 weeks of age to a D% of over 40%, after which they decrease to the levels seen in adults at 8 weeks of age [3, 9]. In the rat pituitary gland, D-Asp is predominantly present in the anterior lobe and its levels increase gradually to a D% of 5% at eight weeks of age [5]. In addition, there is a gender difference in the D-Asp concentrations in the pituitary gland as females have 1.4-fold higher D-Asp levels than males [1].

2. Localization and biological activities of D-Asp in rat tissues

As described above, D-Asp accumulates in particular regions of mammalian tissues and its levels change markedly during development. To define exactly where D-Asp occurs in each tissue, we raised a specific anti-D-Asp antibody for immunohistochemical analysis of the tissues [3-5, 10-12]. This revealed possible biological functions of D-Asp and set the stage for other experiments aiming to determine whether the D-Asp in a particular tissue has been synthesized by the tissue or has been obtained from elsewhere.

2.1. Brain

The quantitative analysis indicated that in several regions of the brain of human or rat embryos, D-Asp levels rise transiently during development and decrease rapidly after delivery [1, 6-9]. Probing of the rat embryonic brain with the anti-D-Asp antibody at different

stages of development revealed for the first time that D-Asp first emerges in the hindbrain, after which it spreads into the forebrain and then throughout the whole brain [11]. Confirming the results of the quantitative analysis, the immunoreactivity (IR) of D-Asp markedly diminishes after parturition [11].

With regard to brain cell-specific expression of D-Asp, D-Asp first occurs in the cell bodies of neuronal cells that had migrated into the outer layer of the neuronal epithelium. Thereafter it appears in the processes of neuroblasts and seems to be localized exclusively in axons once the distinct axon layer had been established. Thus, the intracellular localization of D-Asp in neuronal cells alters during differentiation, especially during the process of neurite extension. This suggests that D-Asp plays a physiologically significant role in the development and neurogenesis of the embryonic rat brain [11].

2.2. Adrenal gland

The quantitative analysis revealed that in the rat adrenal gland, D-Asp levels at one week of age are relatively low but increase markedly at three weeks of age, after which a rapid decline occurs [3, 9]. Immunohistochemical analysis revealed that D-Asp emerges in different cell types at distinct periods during development [3]. At one and three weeks of age, D-Asp IR is intense in the cytoplasm of cells in the zona fasciculate and zona reticularis, the two innermost layers of the adrenal cortex, but is negligible in the zona glomerulosa, the outermost layer of the cortex. However, at eight weeks of age, these cortical IR patterns are reversed as the cytoplasmic IR in the zona fasciculate and zona reticularis cells has disappeared and the zona glomerulosa now shows intense IR. The cortical zones differ in function as the zona glomerulosa secretes mineralocorticoids while the inner zones secrete glucocorticoids. The distinct temporal and site-specific patterns of D-Asp in the adrenal gland suggest that D-Asp is involved in the development and maturation of steroidogenesis in this organ (see Section 2.4). With regard to the adrenal medulla of adult rat (eight weeks of age), D-Asp IR appears in the cytoplasm of adrenaline-storing cells and is negligible in noradrenaline-storing cells [3, 13].

As will be discussed in more detail below, D-Asp appears to be biosynthesized in the mammalian body, although the precise synthetic route remains to be elucidated (see Section 3.1). Recent reports including our own suggest that D-Asp is synthesized in certain specific tissues, released into the vascular system, and then taken up by particular cells of various tissues by the L-Glu transporter, which has an affinity for D-Asp as well as for L-Glu and L-Asp [12, 14, 15]. Several observations suggest that the adrenal gland is one of the tissues that takes up D-Asp rather than synthesizing it [12]. First, D-Asp administered intraperitoneally (“exogenous D-Asp”) is incorporated into the same region of the tissue where “endogenous D-Asp” is found. Second, the transient increase in D-Asp levels during the development of the tissue coincides with a transiently increased expression of the Glu transporter. Third, the Glu transporter in the adrenal gland is found largely on the D-Asp-containing cells.

2.3. Pineal gland

The adult rat pineal gland has very high D-Asp levels and IR analysis revealed a regional difference in that intense IR is evident in the distal (caudal) portion of the gland but little staining is found in the proximal (rostral) region [10]. In addition, D-Asp is concentrated in the cytoplasm of pinealocytes, the cell type that constitutes approximately 80% of the cells in this gland and that synthesizes and secretes melatonin. As the pinealocytes in the distal portion of the gland are believed to be closely involved in the synthesis and secretion of melatonin, the localization of D-Asp in this portion suggests that this amino acid may be involved in this process. Supporting this notion is that cultures of primary rat pinealocytes exposed to D-Asp reduce their norepinephrine-

induced secretion of melatonin in a dose-dependent manner [16]. D-Asp may affect melatonin secretion through the activation of a subtype(s) of L-Glu receptor, because it has an affinity for the receptor [17-19].

Like the adrenal gland, the pineal gland appears to take up D-Asp synthesized elsewhere rather than synthesize it itself. This is supported by the observation that D-Asp synthesis is minimal to non-existent in cultured primary pinealocytes and that these cells are highly efficient in taking up exogenous D-Asp from the medium [16]. Moreover, the pinealocytes express the L-Glu transporter [20].

It has been shown that cultured pinealocytes loaded with D-Asp will release their D-Asp when they are stimulated by norepinephrine [16]. Norepinephrine also induces pinealocytes to secrete melatonin. Thus, the norepinephrine-induced release of D-Asp by pinealocytes may represent a feedback mechanism by which norepinephrine regulates its ability to induce melatonin secretion.

2.4. Testis

A cross-section of the seminiferous tubules of the testis will reveal a few spermatogonia along the basement membrane, one or several layers of spermatocytes further towards the lumen, and a group of spermatids next to the tubule lumen. Thus, as the germ cells progress through the various stages of their development, they move from the basal side of the tubule towards the lumen. There are also interstitial cells outside the seminiferous tubules, such as Leydig cells that synthesize and secrete testosterone (Fig. 2). In the adult rat testis, D-Asp IR is noted in the cytoplasm of germ cells, particularly in the elongate spermatids, the most mature of the germ cells [4]. The IR intensity in each seminiferous tubule differs because each tubular cross-section contains germ cells at a different stage of development. Almost no staining is recognized in cells other than germ cells, including Sertoli cells, Leydig cells, and other testicular cells (Fig 2).

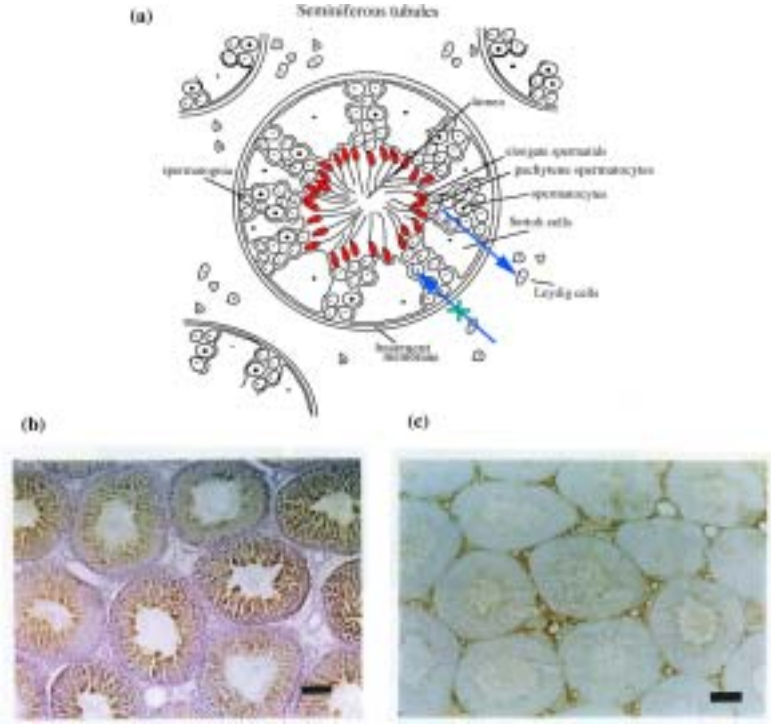

Fig.2 D-Asp in the rat testis
(a) Representative illustration of the adult rat testis. Seminiferous tubules include Sertoli cells and germ cells that are in various stages of their development. D-Asp is primarily localized in the cytoplasm of germ cells, particularly in the region rich in elongate spermatids (shown in red). D-Asp is apparently synthesized inside the seminiferous tubules and secreted out of the tubules into the interstitial space where it stimulates Leydig cells to increase testosterone production by stimulating StAR gene expression. However, exogenously administered D-Asp accumulates in the interstitial spaces of the testis rather than being incorporated into the inside of the tubules (blue arrow). (b) Immunohistochemical staining of D-Asp in the adult rat testis (eight weeks of age) with anti-D-Asp antibody. Immunoreactivity was visualized with the peroxidase-antiperoxidase complex and diaminobenzidine and is therefore shown as brown signals. Counter-staining with hematoxylin and eosin was also carried out. D-Asp is localized in the central areas of the seminiferous tubules, mainly around the region rich in elongate spermatids. Details are described in [4] Bar: 100 μm, anti-D-Asp antibody: 1:100. (c) Adult male rats (eight weeks of age) were injected intraperitoneally with D-Asp (1.0 μmol/g antibody. Exogneous D-Asp (brown) accumulated in the testis by being taken up from the vascular system is primarily localized in the interstitial space and is not incorporated into the seminiferous tubeles. Bar: 100 μm, anti-D-Asp antibody: 1:1000. Details are described in [4].

To confirm these patterns of D-Asp localization in the testis, toxic chemicals were applied to rats to eliminate specific testicular cell populations [4]. For example, administration of methoxyacetic acid causes the specific loss of pachytene spermatocytes and elongate spermatids three and 20 days later, respectively. Three days after methoxyacetic acid treatment, when pachytene spermatocytes are specifically deleted but the elongate spermatid numbers show no significant change, L-Asp levels and total protein levels are reduced but D-Asp levels are unchanged. This is consistent with the immunohistochemical observation that pachytene spermatocytes contain a relatively low amount of D-Asp. In contrast, twenty days after the treatment, when the elongate spermatid numbers are severely depleted, a significant decrease in D-Asp levels is observed together with reduced L-Asp and protein levels [4]. These observations confirm that elongate spermatids contain higher levels of D-Asp than the germ cells at earlier stages of development.

It appears that when the elongate spermatids are released as free spermatozoa into the epididymides to continue their development, they leave their D-Asp behind in the seminiferous tubule, because D-Asp levels in the rat epididymides and epididymal fluid are very low compared with those of the testis [4]. This suggests that most of the D-Asp in the elongate spermatids is left behind in the cytoplasmic fragment remaining in the seminiferous tubules when the cells expel most of their cytoplasm in the tubules and are released into the epididymides. Thus, D-Asp appears to play a significant role(s) in the testis rather than in the spermatozoa.

The germ cells in the seminiferous tubules of the testis, unlike the adrenal and pineal gland cells, appears to synthesize their own D-Asp because intraperitoneally administered D-Asp accumulates in the interstitial spaces of the testis (i.e. outside the tubules) rather than being incorporated into the tubules (Lee and Homma, unpublished observation, Fig. 2). That high D-Asp concentrations are found in rat testicular venous blood plasma [21] support the notion that D-Asp is produced within the seminiferous tubules of the testis and that it is then secreted out of the tubules.

It appears that the secreted testicular D-Asp acts to modulate testosterone synthesis by Leydig cells. Treatment of purified rat Leydig cells with D-Asp directly increase human chorionic gonadotropin (hCG)-induced testosterone synthesis [22]. The increase is dependent upon the culture period with D-Asp, with considerable stimulation being observed after three hr of culturing with D-Asp. Testosterone secretion by Leydig cells is stimulated by D-Asp even in the absence of hCG stimulation, and D-Asp and hCG appear to have synergistic rather than additive effects on testosterone production. This stimulation is independent of the L-Glu receptor on rat Leydig cells, as L-Glu, L-Asp and various synthetic agonists of the receptor do not have the same effect as D-Asp. In addition, Glu receptor antagonists do not suppress the stimulatory effect of D-Asp. However, it appears that rat Leydig cells express a L-Glu transporter subtype denoted as GLAST that may specifically mediate their D-Asp uptake. L-Cysteine sulfinic acid inhibits D-Asp uptake by this transporter and when cultured Leydig cells are treated with this inhibitor, both the uptake of D-Asp and the D-Asp-mediated stimulation of testosterone production is decreased in a dose-dependent manner. The increase in testosterone production also correlates well with the amount of D-Asp taken up. Thus, it appears that Leydig cells take up D-Asp and that this stimulates testosterone production.

The mechanism by which D-Asp increases Leydig cell testosterone production was assessed by examining the effect of D-Asp on various stages of the testosterone synthetic pathway [22]. After hCG challenge of Leydig cells, D-Asp has no effect on the levels of

cAMP, suggesting that D-Asp exerts its effect downstream of the point cAMP is formed (Fig.3). For testosterone synthesis, the process of cholesterol translocation from the outer to the inner mitochondrial membrane is a rate-limiting step and overall rate of the synthesis is limited by the availability of cholesterol to intramitochondrial P450scc (Fig. 3).

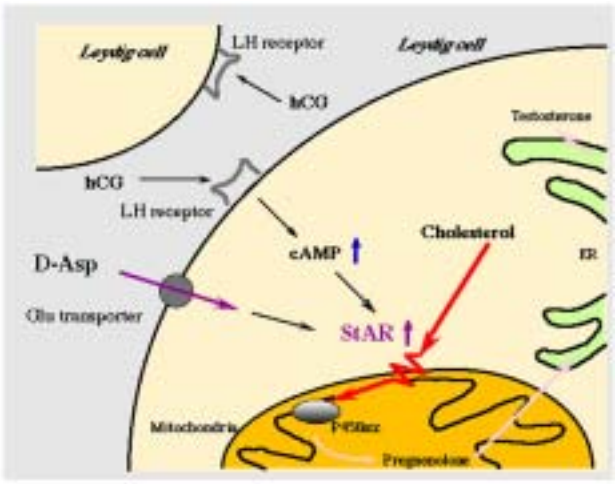

Fig.3 D-Asp action on Leydig cells in the testis.
In Leydig cells, cholesterol is transported across the outer mitochondrial membrane to the inner mitochondrial membrane where it is converted to pregnenolone by P-450scc. Pregnenolone is then converted to testosterone via the steroidogenic pathway in the endoplasmic reticulum (ER). The process of translocation of cholesterol to the inner mitochondrial membrane is the rate-limiting step for testosterone biosynthesis. This step is activated in Leydig cells when human chorionic gonadotropin (hCG) binds to the luteinizing hormone (LH) receptor. Stimulation of this receptor results in increased levels of cAMP, which then stimulates Steroidogenic Acute Regulatory (StAR) protein gene expression. The StAR protein subsequently facilitates the translocation of cholesterol to the inner mitochondrial membrane and increases testosterone production. D-Asp appears to be taken up by the Leydig cells via their L-Glu transporters after which it increases StAR gene expression by an as yet undefined mechanism.

To surmount this limitation, 22(*R*)-hydroxycholesterol, a freely accessible substrate to P450scc can be supplied to measure the ultimate capacity of testosterone production in the cells. In the presence of this cholesterol derivative, D-Asp does not increase testosterone production any more, suggesting that D-Asp accelerates the process of cholesterol translocation to the inner mitochondrial membrane, a key and rate-limiting process in the steroidogenesis. Recently Steroidogenic Acute Regulatory protein (StAR) was demonstrated to be a key regulatory factor in this process and D-Asp is shown to actually enhance the steady state mRNA and protein levels of this factor [23]. Thus, D-Asp appears to increase testosterone production in rat Leydig cells by stimulating StAR gene expression.

It is likely that the D-Asp secreted by germ cells also acts to regulate physiological functions in other tissues (e.g. the adrenal gland) in an endocrine fashion by entering the venous blood and traveling via the vascular system to tissues that can take it up.

2.5. Pituitary gland

The pituitary gland consists of three different lobes, namely, an anterior lobe made up of endocrine cells, an intermediate lobe, and a posterior lobe made up of neuronal axons and glial cells. In the posterior lobe of adult rats, D-Asp IR is spread homogeneously over an area apparently corresponding to that occupied by neuronal axons, while in the anterior lobe intense IR is scattered throughout the cytoplasm of the endocrine cells [5, 13]. HPLC analysis indicates that the D-Asp levels in the anterior lobe are approximately seven-fold higher than those in the posterior lobe [5]. The anterior lobe of the rat pituitary gland is constituted by at least six different cells types. One is the folliculo-stellate cell type and the remaining five types produce and secrete their distinct hormones, namely, growth hormone, prolactin, ACTH, thyroid stimulating hormone, and gonadotropic hormone. Double staining with antibodies against D-Asp and prolactin revealed that the D-Asp-positive cells in the anterior lobe of the rat pituitary gland are prolactin-producing cells or another closely related cell type [5]. This is supported by several observations. First, the morphological features of the D-Asp-positive cells as revealed by electron microscopy are similar to those of prolactin-producing cells. Second, D-Asp levels in the pituitary gland are increased by estrogen

implantation, which is also known to increase prolactin-producing cell numbers and serum prolactin levels. Third, D-Asp levels and prolactin-producing cell numbers are both higher in females than in males [5].

It appears that the anterior lobe of the pituitary gland synthesizes most of its own D-Asp because intraperitoneally administered D-Asp entering the pituitary gland is incorporated mainly into endothelial cells, not into prolactin-producing cells [12]. Furthermore, changes in Glu transporter expression that occur during pituitary gland development do not correlate with development-related changes in D-Asp levels. Moreover, prolactin-producing cells do not express any of the Glu transporters characterized to date. Consistent with these observations is that we found by HPLC analysis and immunocytochemical staining that D-Asp is produced by a prolactin-producing clonal strain of the rat pituitary tumor cell line GH3 [24]. Furthermore, D-Asp dose-dependently enhances thyrotropin-releasing hormone-induced prolactin secretion from the cells [24]. Recent reports also show that isolated pituitary glands or dispersed anterior pituitary cells incubated with D-Asp increase their prolactin secretion [25, 26]. Thus, it appears that the anterior pituitary gland synthesizes D-Asp, which then acts in an autocrine or paracrine fashion to enhance the secretion of prolactin and other hormones.

3. D-Asp in mammalian cells

3.1. Biosynthesis

Until recently, it was not clear whether mammals can synthesize D-Asp since dietary D-Asp is readily absorbed by the intestine and transported via the vascular system into various tissues, where it is taken up by cells carrying the L-Glu transporter [14, 15, 27]. We addressed this issue by employing pheochromocytoma (PC) 12 cells, a cultured mammalian cell line [28]. HPLC analysis, immunocytochemical staining, and use of an enantioselective degradative enzyme (D-Asp oxidase) revealed that PC12 cells contain D-Asp and that the cellular levels of D-Asp increase with the duration of culture. As this amino acid was not added to the culture medium, our observations demonstrate for the first time that D-Asp is indeed synthesized by mammalian cells. Exogenous D-Asp is not taken up into the cells because the cells do not express L-Glu transporter, confirming that the biosynthesis takes place inside the cells. The D-Asp in PC cells constitutes 12-14% of the total Asp and other D-amino acids have not been detected. Rat pituitary tumor cells (GH3) have also been found to synthesize D-Asp [24] (see also Section 2.5), unlike mouse 3T3 fibroblasts and human neuroblastoma NB-1 cells [28].

Although the mammalian D-Asp synthetic pathway is yet to be defined, the enzyme that is most likely to be responsible for its biosynthesis is Asp racemase. Asp racemase from shellfish [29] and archaea [30, 31] has been characterized and genes encoding Asp racemase have been cloned from hyperthermophilic archaea [30, 31]. High concentrations of D-Asp are observed in these living organisms and it is believed the racemases are responsible for this. The enzymes from the hyperthermophilic archaea are Asp-specific and independent of pyridoxal 5'-phosphate but we recently found a different Asp racemase in an acidothermophilic archaeon that is dependent on pyridoxal 5'-phosphate and relatively insensitive to a SH-modifying reagent, unlike the enzymes in the hyperthermophilic archaea [32].

3.2. Mechanisms influencing intracellular D-Asp levels

Elucidating the mechanisms that affect D-Asp levels in mammalian cells will greatly assist the understanding of the physiological significance of D-Asp in the mammalian body. To investigate this issue, we assessed the intracellular localization of D-Asp in 2068 and MPT1 cells, which are variant strains of PC12 cells and whose D-Asp levels are higher than those in PC12 cells [2, 33, 34].

Like PC cells, these strains both synthesize D-Asp. Immunofluorescent staining of 2068 cells with the anti-D-Asp antibody and confocal laser scanning microscopy revealed that D-Asp IR occurs in the cytoplasm, particularly around the nuclei. In addition, there are granular structures bearing fluorescent grains. These observations suggest that D-Asp is associated with granule-like structure(s) in the cytoplasm and that it is released by exocytosis. Consistent with this notion is that D-Asp is released from 2068 cells when the cells are depolarized with a high concentration of KCl or when the cells are incubated with the Ca^{2+} ionophore A23187. Depletion of extracellular Ca^{2+} ions also significantly suppresses D-Asp release [33]. Several reports including our own have also demonstrated that D-Asp is released by cells [16, 35, 36].

It is still unclear at present how cellular D-Asp concentrations are regulated. We have noted that the cellular levels of D- and L-Asp in the variant PC12 cell line MPT1 are regulated by the cell density in the culture because when cell density increases, D-Asp levels increase while L-Asp levels decrease [34]. The D- and L-Asp levels in the cells are dependent on the number of cells inoculated, thus the cellular levels of D-Asp increase, while L-Asp levels decrease in the culture plates, in which higher numbers of cells are inoculated. The effect of cell density on D- and L-Asp levels is also observed when equivalent numbers of MPT1 cells are inoculated in culture plates with different diameters. In marked contrast, however, when PC12 cells are examined, increasing PC12 cell density decreases L-Asp levels but has almost no effect on D-Asp levels. As MPT1 cells express the L-Glu transporter but PC12 cells do not, it appears that the L-Glu transporter is involved in the cell density-dependent control of the D-Asp content in MPT1 cells. It has been reported earlier that several transporter systems of amino acids, including the L-Glu transporter, alter in activity when the cell density changes (see references in [34]). These lines of evidence also show that different mechanisms regulate D- and L-Asp levels in mammalian cells.

Notably, individual PC12 cells incubated with the anti-D-Asp antibody differ in their staining intensity [28]. This individual variation is even more pronounced in 2068 cells and is particularly prominent in round-shaped cells that have condensed chromosomes and appear to be undergoing mitosis [33]. Thus, it appears that cellular D-Asp levels alter during the cell cycle, although this observation requires confirmation and clarification with additional experiments.

Thus, mammalian cells are able to biosynthesize, release, and take up D-Asp. It has also been demonstrated that mammals can degrade D-Asp by the use of D-Asp oxidase, a peroxisomal enzyme that catalyzes the oxidative deamination of D-Asp to generate oxaloacetate together with hydrogen peroxide and ammonium ions. The oxidase prefers acidic D-amino acids such as D-Asp and D-Glu and does not act on L-Asp [37]. A different type of oxidase, D-amino acid oxidase, is also present in the peroxisome, which acts on neutral and basic D-amino acids but not on acidic D-amino acids [37]. These lines of evidence indicate that mammalian cells contain all the molecular components essential for the regulation of D-Asp levels.

4. D-Asp in the mammalian body

In summary, it appears that D-Asp is a novel type of messenger in the mammalian body (Fig. 4) that is synthesized by specific tissues and cells (Section 3.1), including the anterior lobe of the pituitary gland (Section 2.5) and the seminiferous tubules of the testis (Section 2.4). D-Asp is released from the cell after its synthesis (Section 3.2) and may act in an autocrine and paracrine fashion on the synthesizing cell itself and/or neighboring cells. An example of such autocrine activity is that in the anterior lobe of the pituitary gland, prolactin-producing cells synthesize D-Asp that then stimulates these cells to secrete more

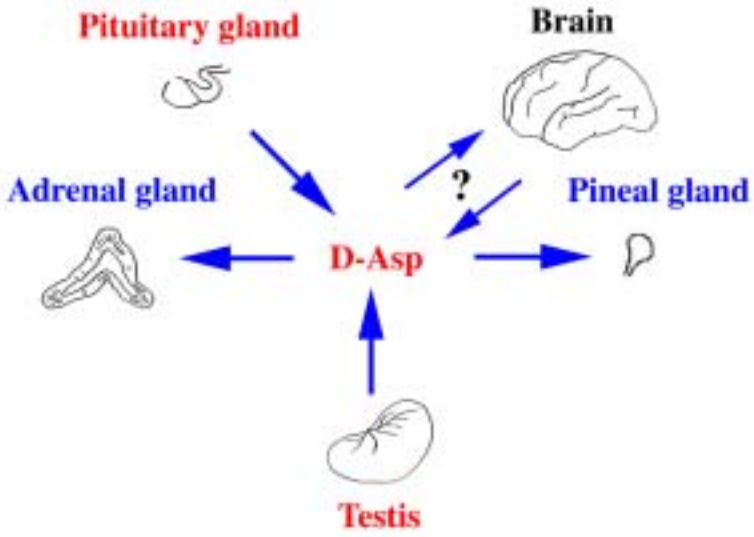

Fig. 4 D-Asp in the mammalian body.
D-Asp is a novel type of messenger in the mammalian body. It appears to be synthesized in the anterior pituitary gland and the seminiferous tubules of the testis where it stimulates the prolactin-producing cells in the anterior pituitary gland to secrete more prolactin and the Leydig cells in the testis to increase testosterone production. D-Asp is probably also released by these tissues and travels via the vascular system to other tissues that take it up via their L-Glu transporters. These tissues include the adrenal and pineal glands and possibly the brain. In the pineal gland, D-Asp suppresses the ability of pinealocytes, the parenchymal cells of the gland, to secrete melatonin. In the adrenal gland, it appears to modulate steroidogenesis. The functional significance of D-Asp in the brain remains to be clarified.

prolactin (Section 2.5). An example of paracrine activity is that D-Asp secreted by the seminiferous tubules may stimulate Leydig cells in the interstitial space to increase their testosterone production by stimulating StAR gene expression (Section 2.4). D-Asp secreted by tissues that synthesize it may also act in an endocrine fashion on other tissues by entering the vascular system and being taken up by cells bearing the L-Glu transporter (Section 3.2). One example of this is testicular D-Asp, which is known to be secreted into the testicular venous blood plasma (Section 2.4). The tissues that take up D-Asp synthesized elsewhere include the adrenal and pineal glands. D-Asp taken up by the adrenal gland appears to modulate the steroidogenesis that occurs in that organ, similar to the way testosterone production by the testis is regulated by D-Asp (Section 2.2). Similarly, D-Asp taken up from the vascular system may suppress melatonin secretion by pinealocytes, the parenchymal cells of the pineal gland (Section 2.3).

The brain contains D-Asp during its embryonic development but as yet the functional significance of this is unclear. However, D-Asp is an agonist for the L-Glu receptor, which may be involved in the development and neurogenesis of the brain. Furthermore, since the brain is a steroidogenic tissue like the testis and adrenal gland [38], D-Asp may modulate the production of neurosteroids in the brain similar to the way this occurs in the latter tissues.

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