PERSPECTIVE

Aromatic L-amino acid decarboxylase cells in the spinal cord: a potential origin of monoamines

Monoamine neurotransmitters include catecholamines and indoleamines. The most common catecholamines are dopamine (DA), noradrenaline (NA) and adrenaline, which are produced from phenylalanine and tyrosine; whereas the most common indoleamine is serotonin (5-hydroxytrypta mine, 5-HT), which is produced from 5-hydroxytryptophan (5-HTP). In the central nervous system, monoamine neurotransmitters come from specific monoaminergic neurons that occur in a variety of brain regions. In the mammalian spinal cord the different monoamine neurotransmitters, which are important modulators of both sensory and motor functions, are commonly believed to originate in different supraspinal brain regions. For example, 5-HT is produced by neurons in the caudal brain stem raphe nuclei, which include the raphe magnus, raphe obscurus, raphe pallidus, ventral lateral medulla and the area postrema; DA is mainly from the hypothalamic A11 region; and NA is mainly from the locus coeruleus. Traditionally, it has been held that monoamine neurotransmitters cannot be produced in the spinal cord itself. However, following spinal cord transection, some 2–15% of the normal complement of 5-HT and about 5% of the normal complement of NA remain in the spinal cord below the lesion (Magnusson, 1973; Schmidt and Jordan, 2002) although the data about the existence of residual DA are inconsistent to date. For decades, neuroscientists – especially those in the field of spinal cord injury (SCI) research – have been frustrated in their efforts to determine the origins of these small amounts of monoamines. Recently, accumulating evidence has suggested that one possible origin might be the intraspinal monoaminergic neurons. Indeed, some intraspinal 5-HT, tyrosine hydroxylase (TH) and DA β-hydroxylase neurons have been found in mammalian spinal cord (Mouchet et al., 1986; Neuton and Hamill, 1988). However, intraspinal monoaminergic neurons are both very few in number and sparsely distributed. For example, the entire rat spinal cord contains only 3–9 5-HT cells, which occur mainly in regions below the cervical spinal level (Neuton and Hamill, 1988); intraspinal DA cells occur mainly at cervical and sacral levels; and intraspinal NA cells occur mainly in cervical segments (Mouchet et al., 1986). Considering these facts, it seems quite improbable that, following complete spinal transection, intraspinal monoaminergic neurons are the sole source of residual monoamines caudal to the lesion. Then what are the other possible sources? Recent findings by our group (Wienecke et al., 2014) and Bennett's group (Li et al., 2014) point to the aromatic L-amino acid decarboxylase (AADC) cells in the spinal cord.

Aromatic L-amino acid decarboxylase is an enzyme that is essential for the conversion of 5-HTP to 5-HT and of L-dopa to DA. It is also involved in the synthesis of trace amines such as tyramine from tyrosine, 2-phenylethylamine from phenylalanine and tryptamine from tryptophan. Previously, AADC cells have been described mainly around the central canal and normally do not contain monoamines (Jaeger et al., 1983). However our findings (Wienecke et al., 2014) and those of Li et al. (2014) have challenged this traditional notion. Using a sacral spinal cord transection rat model the two groups independently found that after SCI, AADC cells in the spinal cord increase their ability to use 5-HT precursor, 5-HTP, to synthesize 5-HT. We showed that AADC cells are not limited to the vicinity of the central canal but occur in several different regions of gray matter throughout the length of the rat spinal cord (**Figure 1A**). We also showed that when comparable amounts of 5-HTP were injected intraperitoneally, only a small proportion of AADC cells in the normal/sham-control rats became 5-HT immunopositive, whereas in the chronic SCI rats almost all of the AADC cells became 5-HT immunopositive (**Figure 1B**, **C**). More importantly, we demonstrated that the increased ability of AADC cells to synthesize 5-HT following SCI is the result of a loss of inhibition by descending 5-HT neurons and that it is mediated by 5-HT1B receptors expressed by AADC cells.

Then, what is the significance of the phenotypic change in AADC cells following SCI? As demonstrated by our results (Wienecke et al., 2014) and those of Li et al. (2014), one important effect of this phenotypic change is to promote the development of spasticity following SCI by causing an increased motoneuronal excitability. Although the pathophysiology of spasticity involves multiple mechanisms, including remodeling of intrinic spinal circuitry, release from presynaptic inhibitions and increased activity of motoneurons and interneurons, one cause for the increased motoneuron excitability is an enhancement of inward persistent currents (Hultborn et al., 2013). It is highly relevant that monoamines and their receptors are among the factors that mediate inward persistent currents. We have demonstrated that following SCI 5-HT2A and 2C receptors are significantly upregulated in motoneurons and/or interneurons (Kong et al., 2011; Ren at el., 2013). This upregulation of HT2 receptors might consequently induce the supersensitivity of motoneurons to 5-HT. As summarized in **Figure 2**, following SCI, the ability of AADC cells to synthesize 5-HT from 5-HTP is increased. When a small amount of 5-HTP is available, *e.g.*, from the cerebrospinal fluid, the AADC cells in the spinal cord below the injury could provide a small amount of 5-HT which in turn acts on motoneurons/interneurons *via* volume transmission or synaptic connections (Wienecke et al., 2014). *Via* upregulated 5-HT2 receptors this could subsequently increase motoneuron excitability and thus cause muscle spasm.

Although we established that AADC cells in the spinal cord could potentially provide 5-HT after SCI, even with the addition of a monoamine oxidase inhibitor, we were unable to detect 5-HT immunoreactivity in AADC cells until exogenous 5-HTP was introduced (Wienecke et al., 2014). Similarly, even using a high-performance liquid chromatographic technique, Li et al. (2014) could not detect the existence of 5-HT at spinal cord levels distal to an SCI without prior 5-HTP application. The question is, then, could AADC cells produce 5-HT without 5-HTP administration? Previous evidence indeed indicates this might be the case. Thus, using a high-performance liquid chromatograph technique Hadjiconstantinou et al. (1984) have shown that following application of pargyline (a monoamine oxidase inhibitor) without concurrent 5-HTP application, 5-HT content in the spinal cord below the transection is increased 8.5-fold,

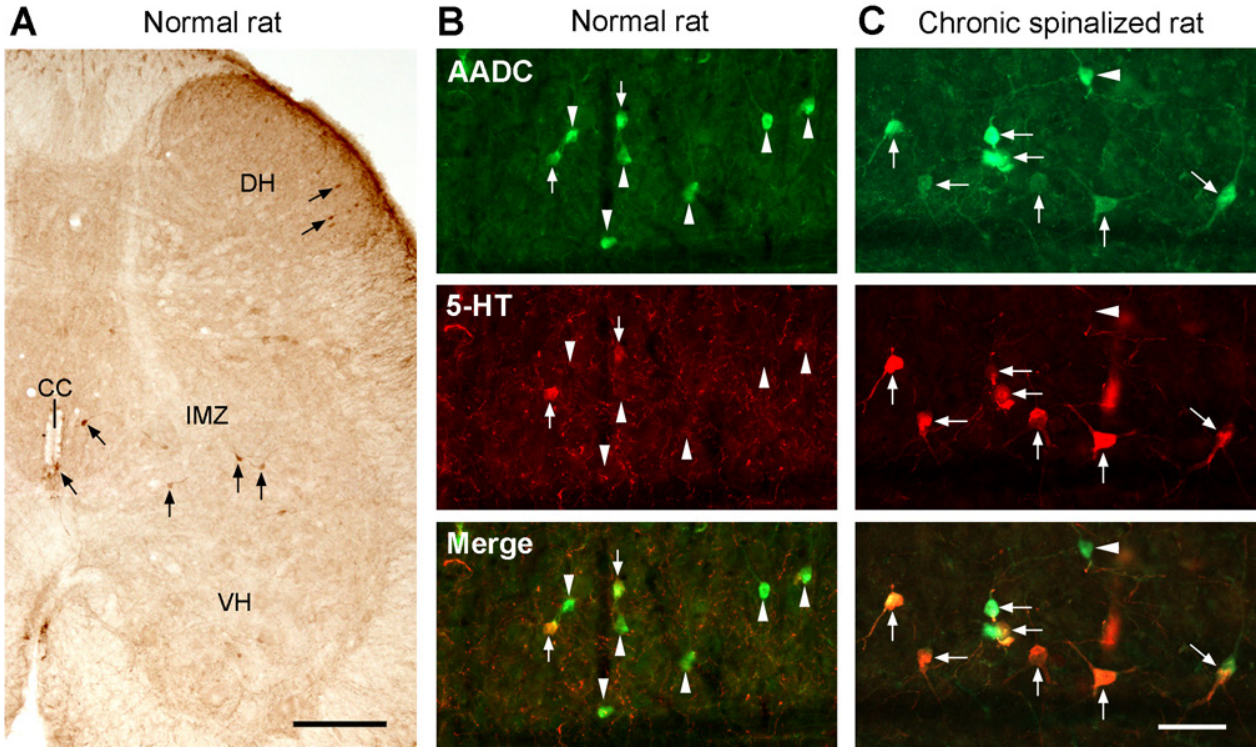

Figure 1 Aromatic L-amino acid decarboxylase (AADC) cells in a normal rat spinal cord and 5-hydroxytryptamine (5-HT) expression in AADC cells in normal and chronic spinalized rat spinal cords after 5-hydroxytryptophan (5-HTP) systemical application.
(A) In a sacral spinal cord section from a normal rat, AADC cells (arrows) were seen to be expressed not only in the region around the central canal (CC) but also in the dorsal horn (DH) and intermediate zone (IMZ). VH: Ventral horn. (B) In a normal control rat, only a few AADC cells became 5-HT positive (arrows), whereas most AADC cells were 5-HT negative (arrowheads) after 5-HTP intraperitoneal injection (100 mg/kg). (C) In a chronically spinalized rat (79-day after SCI), almost all of the AADC cells expressed 5-HT (arrows) following the same 5-HTP application, although occasional 5-HT-negative AADC cell was also seen (arrowhead). Bar in A, 200 μm; in C (valid for B and C), 50 μm. Modified from Figures 2 and 4 in Wienecke et al., 2014 with permission.

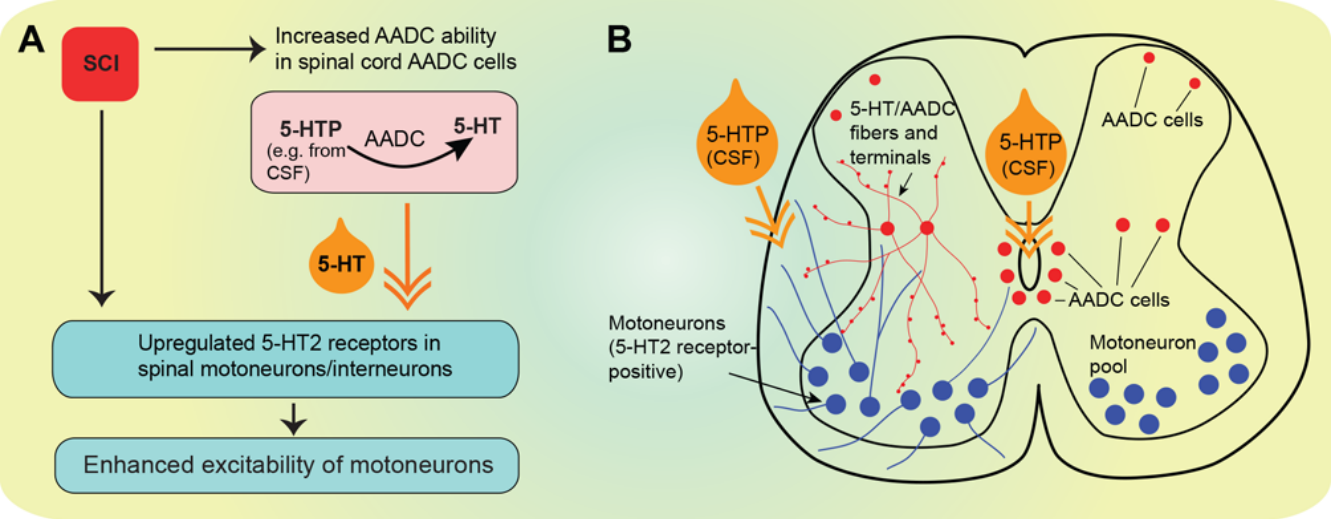

Figure 2 Presumed mechanisms of hyperexcitability of motoneurons induced by 5-hydroxytryptamine (5-HT) produced in aromatic L-amino acid decarboxylase (AADC) cells after spinal cord injury (SCI).
(A) Workflow of plasticity of intraspinal 5-HT system in increasing motoneuron excitability following SCI. Following SCI 5-HT2 receptors (mainly 5-HT2A and 2C receptors) are upregulated in motoneurons and/or interneurons. Meanwhile the ability of AADC cells to produce 5-HT from 5-hydroxytryptophan (5-HTP) is increased following removal of the suppression from serotonergic raphe inputs. Once 5-HTP is available, *e.g.*, from the cerebrospinal fluid (CSF), 5-HT will be produced in the AADC cells and released into extracellular matrix, which in turn acts on 5-HT2 receptors in motoneurons and/or interneurons. These plastic changes in concert will increase the excitability of motoneurons, and thus at least partly underlie the pathogenesis of spasticity after SCI. (B) Schematic drawing of a spinal transverse section illustrating the related anatomical structures in relation to the workflow in A.

whereas the increase was only 4.4-fold in the intact spinal cord. Although the data did not show where this 5-HT originated, it most likely came from the AADC cells. We offer two explanations for the failure of immunohistochemical techniques to detect 5-HT in AADC cells. First, it is quite possible that the concentration of 5-HT produced in the spinal cord below the lesion is too low to be detected by conventional immunohistochemistry. Second, the turnover rate of 5-HT produced in the AADC cells may be so rapid that once produced it is released immediately to the extracellular matrix and this very rapid turnover renders it impossible to detect intracellular 5-HT in the AADC cells. Therefore, what is needed is a more sensitive method, such as fast cyclic voltammetry, which can detect the active release of very small amounts of monoamine neurotransmitters.

To date, the research concerning functions of AADC cells has mainly focused on their production of 5-HT. However, because the enzyme AADC is common to the conversion of 5-HTP to 5-HT and of L-dopa to DA, it is natural to investigate whether the ability of AADC cells to produce DA is also increased. We have a manuscript in preparation that shows this to be the case. Moreover, it is of interest to us that AADC cells could also involve in the production of certain types of trace amines in the spinal cord. Indeed a recent study from Hochman's group (Gozal et al., 2014) indicates that some kinds of trace amines (*e.g.*, tyramine) are expressed in spinal cord AADC cells and trace amine receptors 1 and 4 were also found in the neurons, including motoneurons, of the normal rat spinal cord. Using neonatal rats for an *in vitro* spinal cord preparation, they also showed that tyramine and tryptamine have a direct action on motoneurons, causing them to increase their activity and to generate locomotor-like responses. Although there are no published accounts of trace amines in relation to SCI, it seems obvious that the increased enzymatic activity of AADC cells in the spinal cord following SCI could be expected to generate an increased supply of trace amines which would be expected to exert their own influence on interneurons and motoneurons.

The findings that AADC cells can change their phenotype following SCI may also shed light on a possible mechanism of L-dopa-induced dyskinesia in Parkinson's disease. L-dopa-induced dyskinesia is characterized by abnormal involuntary movements that develop following treatment of Parkinson's disease with L-dopa. On the bases that AADC cells exist in many different brain regions and that AADC is an enzyme that converts L-dopa to DA, we speculate that if AADC activity is increased in Parkinson's disease, then following L-dopa administration, uncontrollable dopamine release will occur in many different brain regions.

In conclusion, the emerging evidence from several research groups, including our own, challenges two traditional notions about monoamine production: first, that monoamines cannot be produced in the spinal cord, and second, that AADC cells in the spinal cord do not produce monoamines. All the emerging evidence suggests that production of monoamines in the spinal cord varies according to prevailing circumstances. Normally, production of monoamines by intrinsic spinal cord neurons is inhibited by monoaminergic projections from the brain. However, once this inhibition is released, *e.g.*, under conditions of SCI, this synthetic capacity is expressed. Revealing the functions of AADC cells in normal spinal sensory/motor control and under pathological conditions following SCI (*e.g.*, spasticity) will no doubt attract more attention in this field. In addition, harnessing the activity of AADC cells in selected brain regions in Parkinson's disease may pave the way to treatment of L-dopa-induced dyskinesia.

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