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High levels of histidine decarboxylase in the striatum of mice and rats

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ABSTRACT

The neurotransmitter histamine is produced in the tuberomamillary nucleus of the posterior hypothalamus; these neurons project broadly throughout central nervous system. Histidine decarboxylase (HDC) synthesizes histamine from histidine; in the brain, its mRNA is expressed exclusively in the posterior hypothalamus. Histamine receptors are expressed throughout the forebrain, including in cortex, hippocampus, and basal ganglia, suggesting functional innervation of these structures. We investigated the distribution of HDC protein in dissected tissue from mouse and rat, anticipating that it would reflect the density of hypothalamic histaminergic axonal projections and thus qualitatively parallel the known distribution of histamine receptors. HDC protein was found at high levels in hypothalamus, as anticipated. Surprisingly, it was found at comparably high levels in mouse striatum. HDC protein was 10-fold lower in cortex, hippocampus, and cerebellum. Specificity of HDC detection by Western blot was confirmed using HDC knockout mice. Similar high levels of HDC protein were found in dissected striatum from rat. Striatum does not, however, contain comparably elevated of histamine, relative to other forebrain structures; we confirmed this fact using HPLC. This discrepancy between HDC protein and histamine levels in the striatum suggests that histamine metabolism and neurotransmission in basal ganglia may have unique characteristics, the details of which remain to be elucidated.

Histamine (HA) is an aminergic neurotransmitter produced by neurons of the posterior hypothalamus [10]. Its functions in the central nervous system, and in neuropsychiatric disease, have been less intensively studied than those of other aminergic neurotransmitters. However, convergent evidence has revealed important roles in the modulation of wakefulness [2,28], appetite and metabolism [14], aspects of cognition and learning [1], and other motivational and behavioral phenomena [10].

All neurotransmitter histamine in the adult central nervous system is believed to derive from a small population of neurons in the posterior tuberomamillary nucleus of the hypothalamus (e.g. [20,29]). These neurons contain histamine, as revealed by specific antisera [10,20], and are the only neurons in the brain that express mRNA for histidine decarboxylase (HDC), which catalyzes the conversion of histidine to histamine [4,15]. Projections from these hypothalamic neurons both throughout the forebrain and to midbrain and brainstem have been documented primarily using anti-histamine antisera. HA-positive fibers have been described projecting to cortex, hippocampus, basal ganglia, thalamus, and virtually all other regions of the central nervous system [10,12,31,32].

The principal histamine receptors in the central nervous system are H1, H2, and H3; a fourth, H4, has been characterized more recently and is expressed most prominently in the spinal cord [25]. H1 and H2 are postsynaptic G-protein-coupled receptors; H3 is primarily presynaptic and modulates transmitter release from both histaminergic and other neurons. These receptors are broadly expressed [10]. The H2 receptor is highly expressed in the striatum, the input nucleus of the basal ganglia, as well as in hippocampus, cortex, amygdala, and other forebrain structures [30]. The H3 receptor is also highly expressed in striatum, as well as in cortex, hippocampus, amygdala, cerebellum, substantia nigra, and cerebellum [22,24]. These receptor expression patterns confirm the

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histaminergic innervation of the striatum revealed by mapping of histamine immunoreactivity, though this innervation of the striatum has not been found to be uniquely dense, relative to other forebrain structures [10].

A recent genetic study of a remarkable family with a high density of Tourette syndrome (TS) demonstrated a rare loss of function mutation in HDC suggesting, for the first time, that disruption of histaminergic neurotransmission contributes, at least in rare cases, to this disorder [7]. TS is a neuropsychiatric condition characterized by stereotyped, semi-voluntary motor or vocal tics, altered sensory processing, and a high comorbidity with obsessive–compulsive disorder [16]. Convergent evidence indicates that functional abnormalities of the striatum, together with some areas of neocortex, are particularly relevant to the development of TS (e.g. [6,21]).

To better characterize histaminergic innervation of the striatum and other forebrain structures in mice and rats, we examined the distribution of HDC protein by Western blotting of dissected tissue with HDC-specific antibody. Since HDC mRNA is produced exclusively in the posterior hypothalamus, HDC protein found in other brain structures is likely to represent presynaptic protein from hypothalamic afferents. As anticipated, we found high levels of HDC protein in hypothalamus and lower levels in hippocampus, cerebral cortex, and cerebellum. Quite unexpectedly, we also found high levels of HDC protein in striatum, comparable to those found in hypothalamus.

Adult male C57Bl/6 mice were used in this study; all animal care and use was approved by and under the supervision of the Yale University Institutional Animal Care and Use Committee. Mice were euthanized by cervical dislocation under anesthesia and their brains rapidly removed and chilled in ice-cold phosphate buffered saline. Target brain regions – hypothalamus, hippocampus, striatum, cerebral cortex (posterior-lateral), and cerebellar cortex – were rapidly dissected and frozen on dry ice.

Knockout mice were used as a negative control for Western blotting. These mice have previously been described [18] and have been used in a number of investigations of the peripheral and CNS effects of histamine. They have been backcrossed to >10 generations onto the C57Bl/6 genetic background. Knockout mice and wild-type controls were produced by breeding heterozygotes in our vivarium; progeny were genotyped by PCR.

Protein was extracted by heating to 90° in aqueous buffer (1× PBS; 1% SDS; protease inhibitor cocktail [Roche]), and was resuspended by sonication. Protein concentration was measured using the bicinchoninic acid (BCA) method (Pierce). 5 μg protein from each sample was run on a 10% SDS-PAGE gel and electroblotted onto nitrocellulose using a Bio-Rad MicroCell Western blotting apparatus. Blots were probed with anti-HDC antibody (Progen Biotechnik, 1:5000) overnight, rinsed with TBS, blotted with secondary antibody (HRP-conjugated goat anti-rabbit, Chemicon, 1:1000), and developed using ECL (Pierce). Membranes were then stripped and re-probed using an anti-GADPH primary antibody (Chemicon). Blots were digitized and quantified using NIH Image. Optical density of the major HDC band at 54 kDa was normalized to GADPH. A confirmatory Western was performed using a different primary anti-HDC antibody (Abcam, rabbit polyclonal anti-HDC) at 0.2 mg/ml.

Mice were deeply anesthetized and euthanized by cervical dislocation. Target brain regions were rapidly dissected on ice and frozen on dry ice. Brain samples were sonicated (3–4 s, on ice) in 20 volumes of a solution containing 1.0% ascorbic acide, 0.25% $HClO_4$, 0.25% $Na_2S_2O_5$, 0.25% disodium EDTA, and 25 ng/ml of α-methylhistamine as an internal standard. 0.1 volume of cold 3.5 M $HClO_4$ was then added; samples were kept on ice for 10 min, centrifuged at 5000 × *g* for 5 min, and supernatant stored at −80 °C until analyzed by high performance liquid chromatography (HPLC).

Histamine levels in these supernatants were determined using a modification of published methods [8,33]. The HPLC system consisted of a Shimadzu 10AD pump (primary), Rheodyne injector, Supelcosil DB-18 5 μm 150 mm × 4.6 mm reverse phase column and a Shimadzu RF-20Axs fluorometric detector (excitation 350 nm, emission 450 nm). A mobile phase (pH 4.6) consisting of 0.16 M KH_2PO_4, 0.1 mM Na octanesulfonic acid, and 0.1 mM disodium EDTA was delivered at a flow rate of 1.0 ml/min. The eluent was mixed with o-phthaldehyde (OPT) derivatizing solution (1.0 ml/min, 0.008% OPT in 0.15 M NaOH, kept on ice) using a T-junction and a post-column reaction coil (1.1 mm OD, 0.55 ID, 100 cm stainless steel tubing). Column, T-junction and coil were kept at 45 °C. This system achieved a detection limit (S/N = 2) of 300 fg histamine. Intra- and inter-assay coefficients of variation for histamine measures in brain samples were 0.9–5.3% and 4.6–5.3%, respectively.

Western blotting using an anti-HDC antibody showed a single major band at 54 kDa, corresponding to the predicted molecular weight of processed HDC protein (Fig. 1A). This 54 kDa protein is processed from a 74 kDa polypeptide chain [11]; a faint band at 74 kDa was visible with longer exposures (not shown).

As predicted from the known mRNA expression pattern, high levels of HDC immunoreactivity were found in hypothalamus; immunoreactivity was ~10-fold lower in hippocampus, neocortex, and cerebellum (Fig. 1B). Similarly low levels of HDC immunoreactivity were found in thalamus in a few experiments in which it was included (data not shown.) Surprisingly, HDC levels in dissected striatum were high, comparable to those in hypothalamus. This pattern was observed in each of 11 mice examined in 3 independent experiments and was highly statistically significant: HDC immunoreactivity was not significantly different in hypothalamus and striatum (paired *t*-test: $p = 0.11$) but was significantly higher in these two brain regions than in all others tested (paired *t*-test; all $p < 0.00001$). An identical result was seen with a distinct polyclonal anti-HDC antibody (Abcam; data not shown).

Antibody specificity was confirmed by doing a parallel Western blot using an HDC knockout mouse [18]; (Fig. 1A). This is particularly important as cross-reactivity between HDC and the closely related aromatic acid decarboxylase (also termed DOPA decarboxylase, DDC) has been reported [3]. Indeed, we found both commercial anti-HDC antibodies described here to cross-react with DDC in immunohistochemical experiments (data not shown). In Western blot, however, the prominent 54-kDa HDC-immunoreactive band vanished in HDC-KO mice. A trace of HDC immunoreactivity remained in the hypothalamus of the knockout mouse; this may indeed represent a low-level of cross-reactivity with DDC, which has a similar molecular weight, but it represents less than 5% of the total immunoreactivity seen in the wild-type mouse.

To check the generality of this surprising result, we examined HDC immunoreactivity in the same brain regions dissected from a Sprague–Dawley rat. An identical pattern, with levels of HDC protein in striatum comparable to those seen in hypothalamus, and dramatically higher than those found in other forebrain regions or cerebellum, was observed (Fig. 1C).

This high level of HDC observed in rodent striatum contrasts with previous reports that tissue histamine content in striatum is comparable to that in other forebrain tissues, and lower than that observed in hypothalamus (e.g. [19,27,29]). We examined tissue histamine content using HPLC in dissected brain regions of wild-type C57Bl/6 mice ($n = 1$–3 per brain region) and wild-type littermate controls from our knockouts ($n = 2$ per brain region). As expected, histamine levels were highest in hypothalamus (Fig. 2). They were approximately an order of magnitude lower in the striatum and other brain regions examined.

Motivated by the recent finding of a dominant loss-of-function mutation of the HDC gene linked with a rare Mendelian form

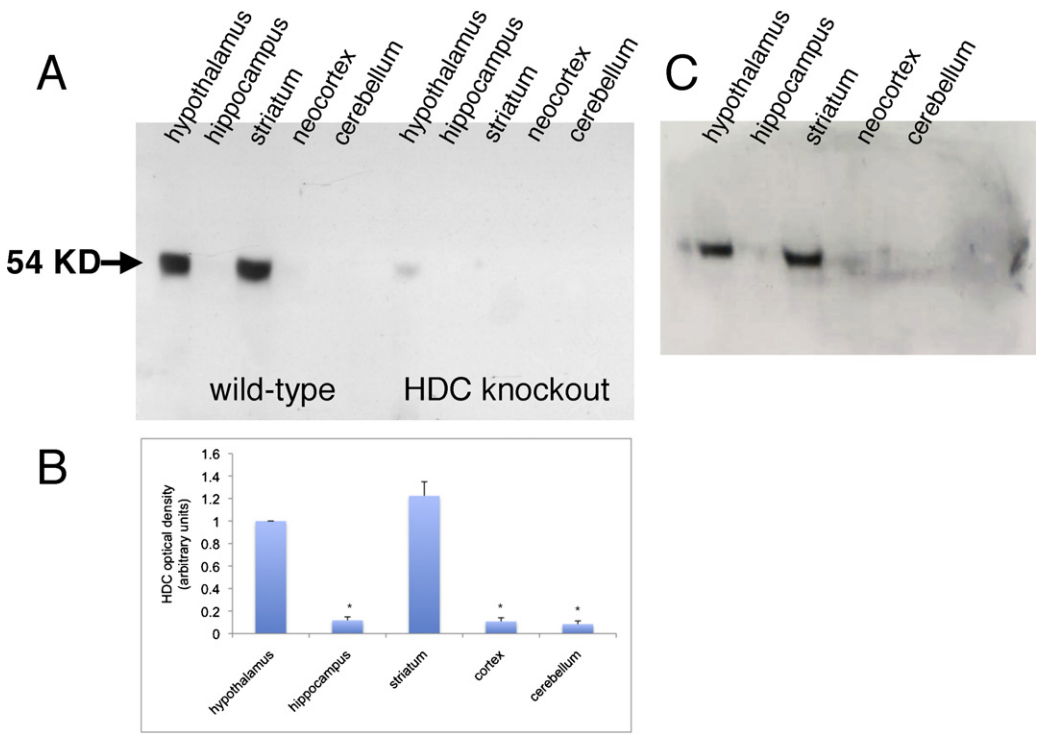

Fig. 1. High levels of HDC protein in mouse and rat striatum. (A) Western blotting for HDC protein in dissected mouse brain tissue revealed a high level of HDC in striatum, comparable to that seen in hypothalamus. Antibody specificity was confirmed by loss of this band in a knockout mouse. (B) Quantification of HDC immunoreactivity confirmed this result ($n = 11$); see text for statistical analysis. (C) A similar analysis in dissected tissue from a Sprague–Dawley rat showed the same pattern of HDC protein in these brain structures.

of Tourette syndrome [7], we examined HDC protein levels in hypothalamus, striatum, and other regions of mouse and rat brain by Western blotting. As expected, we found high levels of HDC protein in hypothalamus, where the mRNA is expressed in histaminergic cells of the posterior tuberomamillary nucleus [4,10,15]. Unexpectedly, we found comparably high levels of HDC protein in the striatum. Levels of HDC protein were approximately 10-fold lower in the other brain regions examined: hippocampus, cerebral cortex, and cerebellar cortex. Since HDC mRNA is not expressed in striatum, we conclude that this high level of striatal HDC derives from projections from cell bodies in the hypothalamus.

Histaminergic innervation of the striatum is well established [10]. Histamine-immunoreactive fibers have been observed in the basal ganglia (e.g. [29]). In addition, the H2 [30] and H3 histamine receptors [22,24] are prominently expressed in the striatum. However, neither histamine itself nor its receptors have been reported to be present at qualitatively higher levels in striatum than in other forebrain structures. The presence of HDC in striatum at levels 10-fold higher than in hippocampus, neocortex, and cerebellar cortex is therefore surprising. This is particularly true since histamine has not been reported to be elevated in striatum, relative to other forebrain structures (e.g. [19,27,29]), a result that we confirmed by HPLC of dissect brain regions from mouse (Fig. 2).

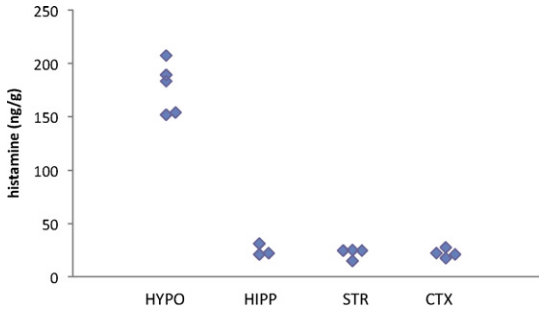

Fig. 2. Histamine is not elevated in striatum proportionally to the level of HDC protein. Histamine was quantified in dissected tissue using HPLC. Hypothalamus HA was higher than all other tissues. Levels in the striatum were not significantly higher than in other tissues, despite the high level of HDC protein in this structure.

Several lines of evidence suggest that histamine processing may be different in rodent striatum than in other brain regions. An early study of HDC activity in different brain regions of rat found 1.4–2.7× greater HDC activity in striatum than in hippocampus or neocortex, though approximately 3-fold lower than in hypothalamus [5]; this is consistent with the high level of protein we observe. A subsequent study in rat found higher histamine turnover in striatum than in all other brain regions examined, apart from hypothalamus [19]. The generality of this result remains unclear, however: a study of regional variations in histamine turnover in ddY mice and in guinea pig did not show higher histamine turnover in striatum than in other forebrain structures [17]. In Ws/Ws rats, which lack mast cells but retain CNS histamine, histamine levels are more profoundly reduced after treatment with the HDC inhibitor fluoromethyhistamine than in other forebrain structures, consistent with a higher rate of breakdown [27]. However, basal histamine release rate was found to be similar in rat striatum and cortex in recent microdialysis studies (e.g. [9]). Therefore, while some early studies noted above, together with our data, suggest that histamine may be processed differently in striatum than in other forebrain structures, the details of any such difference remain to be clarified.

Our results are, of course, dependent on dissection of the defined brain regions, and their spatial resolution is therefore limited by the precision of this dissection. Dissection of striatum was done using technique developed and validated in a previous study in trans-

genic mice [23]. Contamination with a small percentage of globus pallidus, amygdala, or basal forebrain tissue is possible, but contamination with thalamic or hypothalamic tissue can be excluded, as these structures are separated from striatum by the third ventricle and by clean tissue planes. We can therefore be confident that the high levels of HDC protein observed in striatum are not a consequence of contamination with hypothalamic tissue. We cannot formally exclude the possibility that the high levels of HDC seen in striatum derive from mast cells, which are found both perivascularly and parenchymally in the central nervous system, rather than from hypothalamic projections neurons. However, this would imply a markedly elevated number of these cells in striatum, relative to the other brain regions examined, and no such preferential localization has been described (reviewed in, e.g. [26]).

At least one set of early studies used immunohistochemical methods, with a different antiserum, to examine HDC distribution in rat brain [12,31,32]; in these studies, elevated levels of HDC-positive fibers in striatum, relative to cortex, hippocampus, and cerebellum, were not described. There are several possible explanations for this discrepancy. First, details of the specific antisera used and the immunohistochemical methodology may have influenced the sensitivity of detection in these early studies. Indeed, refinement of immunohistochemical technique in the later study in this series [12] revealed a qualitatively different and quantitatively denser network of histaminergic fibers than was apparent in the original studies with this antiserum [31,32], and it is possible that further details of the histaminergic innervation of the striatum remained undetected. The use of a knockout mouse as a control in the current study allows us to be confident that the overwhelming majority of the immunoreactivity we are detecting by Western blot does correspond to HDC.

Second, these earlier studies reported the density of histaminergic fibers; but an increased amount of HDC protein in striatum relative to other structures examined here could correspond to a difference in axon morphology rather than fiber density. Histaminergic fibers are characterized by prominent varicosities [10]; a difference in frequency or size of these varicosities could account for increased HDC in striatum, relative to other structures, but would be undetected by an immunofluorescent analysis of fiber density. Similarly, an increase in the HDC concentration within individual fibers would not be detected in an analysis of fiber density. Under these scenarios, the technique used here – Western blotting in dissected tissue for quantitative detection of whole-tissue HDC immunoreactivity – may be providing complementary information to that provided by immunohistochemical analysis.

The function and effects of histamine in the striatum remain unclear. The recent finding of HDC deficiency in a family with Tourette syndrome [7], a disorder characterized by striatal abnormalities [6,21], adds a new dimension of interest to this already intriguing question. As noted above, H2 and H3 receptors are prominent in this structure [22,24,30]. Interactions between histamine and dopamine, which densely innervates the striatum and critically regulates its information processing, have been a focus of recent interest, motivating exploration of histaminergic agents in the treatment of schizophrenia [13] and as cognitive enhancers [34]. Clarifying histamine–dopamine interactions in the striatum may prove to be critical to a fuller understanding of dopamine dysregulation in a variety of conditions. The finding of exceptionally high levels of HDC protein in the striatum is particularly intriguing in this context.

In summary, we describe a surprisingly high level of histidine decarboxylase protein, as detected by Western blotting in dissected tissue, in mouse and rat striatum. Striatal HDC is comparable to the level found in hypothalamus and far higher than that observed in hippocampus, neocortex, or cerebellar cortex. This finding was robust across multiple replications in mouse and was also observed in rat. Specificity of the detected immunoreactivity was confirmed using a knockout mouse. Elucidation of the functional role of this high level of HDC protein in the striatum is an important area for future research.

Disclosures and contributions

The authors have no real or potential conflicts of interest to declare. KK designed and performed experiments. AGE-S and MX assisted with tissue preparation and Western blotting experiments. HO provided knockout mice. GMA developed and performed HPLC assays of histamine. MWS assisted in design and interpretation of all experiments. CP designed and interpreted experiments, performed data analysis, and wrote the paper; all authors reviewed and edited the manuscript.

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