11β-Hydroxysteroid dehydrogenase 2 vs. transgene: discrepant loci of expression in the adult brain

Joel C. Geerling and Arthur D. Loewy
Department of Anatomy and Physiology, Washington University School of Medicine, Saint Louis, Missouri

TO THE EDITOR: Naray-Fejes-Toth and Fejes-Toth (5) have described the anatomic distribution of Cre-expressing cells in a new strain of transgenic mice. In these mice, the expression of Cre recombinase was targeted to cells that produce an enzyme, 11β-hydroxysteroid dehydrogenase type 2 (HSD2), the original characterization of which benefited greatly from the fundamental contributions of these investigators (4, 6, 7). As this glucocorticoid-inactivating enzyme is necessary for mineralocorticoid receptor (MR)-expressing cells to respond to changes in the concentration of aldosterone within its physiological range (2, 4), a mouse in which Cre is only produced by HSD2-expressing cells would represent a powerful tool for studying the relatively small subpopulations of aldosterone-sensitive cells in organs such as the kidney and the brain.

The primary focus of this article was to show the locations of Cre expression in various tissues, so the Cre-HSD2 mice were crossed with a lacZ reporter strain (10) and X-gal staining was used to visualize cells with transgenic β-gal activity. In the kidney, the cell-type specificity of transgene expression was confirmed; X-gal staining selectively colocalized with immunolabeling for HSD2 and other markers of aldosterone-sensitive epithelial cells in the distal nephron (see Fig. 3 in Ref. 5).

In the brain, however, transgene expression was not analyzed with a similar degree of rigor. No data are provided regarding the colocalization (or lack thereof) of transgene expression with actual HSD2 expression anywhere in the brain. This is unfortunate because although little controversy remains over which parts of the nephron are aldosterone sensitive, existing reports differ regarding the locations of such cells in the brain. Rather than acknowledging and addressing this issue, the authors instead chose to disregard prior data that were not consistent with their interpretation. In particular, they failed to acknowledge the original investigators who revealed the highly restricted distribution of HSD2 expression in the rodent brain using in situ hybridization to detect mRNA for this enzyme (9), as well as a more recent neuroanatomic study demonstrating the exact same distribution via immunohistochemical detection of HSD2 protein (3).

Both of these studies provided unambiguous photographic documentation of the three primary loci of HSD2-expressing cells in the rodent brain: the nucleus of the solitary tract, the subcommissural organ, and the ventrolateral subdivision of the ventromedial nucleus of the hypothalamus. In another study, a weak signal in situ hybridization signal was also reported within the amygdala and locus coeruleus (8). None of these sites are depicted in the study of Naray-Fejes-Toth and Fejes-Toth (5). Instead, only a single brain photomicrograph is provided (Fig. 4C), showing widespread transgene expression in the cerebellum, a structure that contains no detectable HSD2 protein or mRNA in adult animals (3, 8, 9). Furthermore, the subjective intensity ratings provided in Table 1 indicate that transgene expression was found in a potpourri of similarly unexpected brain sites, including the external cuneate nucleus (rated “+++++++”), pontine nuclei (“+++++++”), “Hypothalamic region” (“+++/++++”) and “Thalamic nuclei” (“+++/+ ++”).

A likely explanation for these profound discrepancies is that most Cre-mediated recombination in the brain occurred early in development, in a pattern reflecting the more widespread HSD2 expression found in embryos (1, 8). Most of this developmental HSD2 expression is only transient and disappears shortly after birth in the brain and certain other tissues (1, 8). Even transitory expression of Cre recombinase, however, will permanently activate transgene expression, which will then persist in cells that stop expressing HSD2 (and in any of their mitotic progeny). Given the extensive perinatal pruning of HSD2 expression (1, 8), most of the transgene expression in a mature mouse would then demarcate cells that no longer express HSD2 and are, therefore, no longer protected from glucocorticoids.

Thus, in tissues like the brain, the Cre-transgenic mice developed by Naray-Fejes-Toth and Fejes-Toth (5) may not be useful for selectively studying aldosterone-sensitive cells in a mature animal. Demonstrating Cre or HSD2 expression in the brains of adults from this strain could help in evaluating this possibility. Alternatively, these mice may prove useful for studying the role of HSD2 during development. For example, protection from glucocorticoids may be uniquely important for the development of many of the hindbrain sites listed in Table 1 (external granule layer of the cerebellum, pontine nuclei, reticular formation, cochlear nuclei, lateral reticular nucleus, external cuneate nucleus), which share a common origin in the proliferating neuroepithelium of the rhombic lip (11).

In summary, although the major anatomic discrepancies listed here were neither acknowledged nor addressed by Naray-Fejes-Toth and Fejes-Toth (5), their article should not be misinterpreted as evidence for a broader pattern of HSD2 expression in the brain than that supported by existing neuroanatomic data (3, 8, 9). The formidable technical challenge of creating such a potentially powerful transgenic tool does not justify a disregard for clear-cut inconsistencies between transgene expression and an established pattern of gene expression in normal animals.

REFERENCES


