Appendix VIII

Locations of renal anti-AE4 immunoreactivity (see Section VI)

Reports concerning the renal distribution of AE4 appear to vary depending upon the species being considered and the antibody used to probe AE4 immunoreactivity. In this appendix we consider the findings of each of these studies in isolation. In the review we hypothesize that most of the findings of these studies are consistent with the presence of AE4 in the basolateral membranes of both β- and non-α/non-β intercalated cells in the collecting duct of rats and mice. However, the apparent distribution of AE4 is different in rabbits.

Evidence presented in support of the presence of AE4 at the basolateral membrane of mouse α-intercalated cells. In immunohistochemical studies of mouse collecting ducts (6), two polyclonal anti-AE4 antibodies—raised against non-overlapping sequences in the Ct of rat AE4—stained the basolateral membranes of a subpopulation of epithelial cells. No evidence was provided that AE4-positive cells were also H-pump or AE1 immunoreactive in mice thus, based on this evidence alone, it is not possible to assign AE4 to a specific subtype in mice.

Evidence presented in support of the presence of AE4 at the basolateral membrane of mouse β-intercalated cells. In immunohistochemical studies of mouse collecting ducts, AE4 protein was detected in the basolateral membranes of intercalated cells that expressed pendrin their apical membranes (1). However, pendrin immunoreactivity is not exclusive to β-intercalated cells, as pendrin is also located in non-α/non-β-intercalated cells (5, 9, 11) a cellular subtype that one study found to be as common as β-intercalated cells in the collecting ducts of mice (4). Thus—without a demonstration of an exclusively basolateral H-pump distribution in AE4 and pendrin positive cells—the assignment of β-intercalated cells as a site of AE4 expression in mice is premature. A more recent study harnessed the AE4 promoter to drive the expression of a reporter gene (3). For the most part the expression of the reporter matched that of AE4 itself. The reporter was expressed in a subset of cells that expressed pendrin, but not AE1. This observation was presented in support of the hypothesis that the AE4 promoter drives β-intercalated-cell specific transcription, but without information about the polarized localization of the H-pump in reporter positive cells, it is equally likely that the AE4 promoter is active in non-α/non-β–intercalated cells (3). Praetorius and coworkers also demonstrate AE4 immunoreactivity in the basolateral membranes of mouse collecting duct epithelia, but do not identify the subtype of the stained cells (7).

Evidence presented in support of the presence of AE4 at the apical and lateral membranes of rabbit α-intercalated cells. In a study by Ko et al (6), an anti-AE4 antibody raised to a sequence within the C-terminal domain of rat AE4, detected AE4 immunoreactivity in the lateral and apical membranes of those rabbit collecting duct cells that also expressed an apical H-pump. However, apical H-pump immunoreactivity is common to both α-type and non-α/non-β-intercalated cells and one study found that as

few as 4% of intercalated cells in the rabbit collecting duct may actually be α-types, compared to 57% that are non-α/non-β cells (2). Thus—without a demonstration of the basolateral presence of AE1 in cells with an apical presence of AE4 and H-pump—the assignment of β-intercalated cells as a site of AE4 expression in rabbits is premature.

Evidence presented in support of the presence of AE4 at the apical membrane of rabbit β-intercalated cells. Using polyclonal antibodies raised against the extreme Ct of AE4, two groups of researchers (10, 12) reported the localization of AE4 to the apical membranes of those intercalated cells that stain positive for peanut lectin[1]. A third, preliminary study, reports the presence of AE4 in an unusual, barrel-shaped, subapical structure in a population of intercalated cells that lack AE1 but that stain positive for peanut lectin (8). However, the nomenclature of rabbit intercalated cells is complicated due to the presence of hybrid cell-types such that peanut-lectin-positive cells might be defined as either β- or non-α/non-β intercalated cells (2).

Evidence presented in support of the presence of AE4 in the basolateral membranes of rat α-intercalated cells. An immunohistochemical study by Ko et al (6) using a polyclonal antibody generated against the Ct of rat AE4 detects AE4 immunoreactivity in the basolateral membranes of those cells in the collecting duct that stain for the H-pump in their apical membranes, which for the reasons discussed above could either be α-intercalated cells or non-α/non-β intercalated cells. Thus the assignment of rat α-intercalated cells as a site of AE4 expression is premature.

Evidence presented in support of the presence of AE4 in the basolateral membranes of rat β-intercalated cells. The same study that reported AE4 expression in the basolateral membranes of rat α-intercalated cells also detected basolateral AE4 immunoreactivity in cells that stained for the H-pump in their basolateral membranes (6), i.e. those that are readily identified as β-intercalated cells. Praetorius and coworkers also demonstrate AE4 immunoreactivity in the basolateral membranes of rat collecting duct epithelia, but do not identify the subtype of the stained cells (7).

[1] Tsugenawa and coworkers report staining only in a subpopulation of peanut lectin positive cells (10).

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