

GNAS: a new nephrogenic cause of inappropriate antidiuresis.

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Nephrogenic syndrome of inappropriate antidiuresis (NSIAD) is the mirror image of nephrogenic diabetes insipidus (NDI): in NDI, the kidneys cannot concentrate the urine, whereas in NSIAD urinary dilution is impaired, independent of the presence or absence of vasopressin. Consequently, patients with NDI are at risk of hypernatremic dehydration, whereas hyponatremia is a typical manifestation of NSIAD, mimicking the syndrome of inappropriate antidiuresis (SIADH).¹

The diagnostic pathways also mirror: In NDI an agonist for the vasopressin V2 receptor (AVPR2), such as D-amino D-arginine vasopressin (DDAVP), is given to assess the ability of the kidneys to concentrate the urinary. Conversely, administration of an AVPR2 antagonist, such as tolvaptan provides an assessment of urinary dilution capacity in patients suspected of NSIAD. In patients who did not present with dysnatremia, yet are suspected of having an underlying defect in urinary concentration, a water deprivation (NDI) or water load (NSIAD) challenges the kidneys for an appropriate response. Lastly, vasopressin levels, measured either directly or indirectly via copeptin² can help distinguish nephrogenic disorders of urinary concentration from those of disturbed vasopressin secretion.

Genetic studies identified AVPR2, a G-protein coupled receptor (GPCR) expressed on the basolateral aspect of the principal cells in the collecting duct as a key molecule in both disorders. This provided an elegant explanation for the clinical observations: recessive loss-of-function mutations in AVPR2 cause NDI, whereas NSIAD is due to dominant gain-of-function mutations. Two recent studies have identified mutations in the stimulatory G-alpha protein GNAS as another cause of NSIAD. The association of GNAS with NSIAD provides further insight into the complex function of GNAS but fits perfectly with our current understanding of the physiology of urinary concentration.

Miyado *et al.* are reporting in this issue of the journal³ two families with a dominantly inherited form of NSIAD segregating with the GNAS variants p.F68_G70 del and p.M255V, respectively. The Gas mutation p.F376V was reported in two unrelated patients with hyponatremia and was associated with additional clinical symptoms suggesting gain-of-function (GOF) not only of AVPR2, but also of other GPCR, including the lutropin (LHGR) and parathyroid hormone (PTH1R) receptors⁴. The severity of the phenotype in the patients with the dominantly inherited mutations was quite variable: some patients presented with seizures in early childhood, associated with euvolemic hyponatremia, inappropriately elevated urine osmolality and suppressed vasopressin levels. Other patients had no apparent symptoms, were normonatremic when investigated, yet had an elevated urine osmolality despite suppressed vasopressin levels and had a history of spontaneously low fluid intake. Symptomatic family members were treated with fluid restriction with normalisation of hyponatremia.

The patients with the spontaneous mutation both presented with hyponatremia in the neonatal period.⁴ One of these was investigated in more detail at the age of three years. Treatment until then had consisted of salt supplementation which was associated with hypertension, consistent with the concept that hyponatremia was due to water overload, rather than salt deficiency. When the salt supplements were stopped at the age of 3 years, blood pressure normalized without further anti-hypertensive medication and the patient remained normonatremic. This is consistent with the idea of early childhood as the “vulnerable” period for NSIAD due to the coupling of fluid and caloric intake. At the age of 3 years, the presumably suppressed thirst was sufficient to maintain normonatremia. Spontaneous urine osmolalities were consistently elevated (800-1000 mOsm/kg) despite suppressed copeptin levels and urine osmolality did not

decrease after tolvaptan, suggesting that urinary concentration was independent of AVPR2 activation by vasopressin. Together, these data clearly establish the diagnosis of NSIAD.

Heterotrimeric G proteins function as molecular switches in signal transduction pathways (Figure 1). Each G protein is composed of an alpha, beta, and gamma subunit (Fig 2 a), encoded by separate genes, and is defined by its α -subunit, which binds guanine nucleotides and interacts with specific receptors and effectors.

Due to its interactions with multiple different GPCR, GNAS has been associated with a whole spectrum of different diseases. Online Mendelian Inheritance in Man (OMIM) currently lists 8 different phenotypes under the GNAS entry 139320, not yet including the two recent reports with NSIAD discussed here. The variability in phenotype partly reflects the nature of mutations (gain- vs loss-of-function), but also the fact that GNAS is highly imprinted, so that in various tissues either the maternal or the paternal allele can be predominantly expressed.⁵

Existing structural models of GNAS homologs provide some insight into the potential disease mechanisms (Figure 2). The G α s subunit is composed of a Ras (Rat sarcoma)-like GTPase domain and an α -helical domain (Figure 2a). The GDP (guanosine diphosphate) binding site occupies the interface between these two domains. F376 belongs to the α 5 helix of the G α subunit (Figure 1), which is critical for the receptor-induced release of the nucleotide from the G protein. The F376V mutation most likely induces a conformational change in the G-protein, that in wild-type protein is induced by activation of the associated receptor, explaining the receptor-independent signaling that is reflected in the high urinary concentration of the patient even when the receptor is blocked with tolvaptan (Figure 2b). The resistance of hyponatremia to Tolvaptan is consistent with Gs activity independent from AVPR2

(illustrated in the top panel of Fig 2b). Tolvapan Inhibition of Gs (activated by AVP as illustrated in the bottom panel of Fig 2b) is not visible in vivo probably because of the signal amplification from Gs to AC to PKA to aquaporins. The differences observed in the basal activation of F376V between conditions in which different GPCR were coexpressed in vitro could come from coupling properties of each receptor as well as from the possibility that they also couple to Gi protein (modification of cAMP accumulation). In this case in vitro data are hard to interpret.

M255 localises to the Ras domain of the α subunit, but interacts with N167 in the helical domain, suggesting that the M255V mutation destabilises the closed conformation, enhancing nucleotide release. In contrast, F68-70 are part of the helical domain, but the deletion mutation presumably also destabilise the closed conformation (Figure 2c).

Conclusion

Deciphering rare cases of hereditary hyponatremia is a rich source for understanding GPCR signaling and human physiology.

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Figure legends

Figure 1 : A schematic representation of the structure of the beta 2adreno-receptor–Gs Complex using the 3SN6 pdb file⁶. F376 belongs to the alpha 5 helix of the G alpha subunit which is critical for the receptor-induced release of the nucleotide from the G protein. M255 belongs to the Ras domain of the alpha subunit and F68-70 to the HD domain.

Figure 2a : Proposed mechanism of receptor-catalyzed nucleotide release (from⁷). **(Left)** The Ras and helical domains (Ras and HD) separate frequently, even in the absence of a receptor, but such separation does not usually lead to GDP release. This rapid (relative to overall GDP release) equilibrium favors the closed conformation (top). **(Middle)** Binding of an activated receptor (R*) favors a Ras domain conformational change—displacement of $\alpha 5$ away from GDP—that weakens interactions between GDP and the Ras domain, allowing GDP to escape when the G α domains happen to spontaneously separate (bottom). **(Right)** Loss of GDP shifts the equilibrium toward G α conformations with widely separated domains (bottom). From⁷ with permission.

Figures 2b and c: Proposed mechanism of gain of function with G α s mutations F376V, M255V or del 68-70. (using the same format as in panel a).

Panel b: the F376V $\alpha 5$ mutation leads to the destabilization of the C-terminal helix (middle panel in red) thus mimicking the binding of an activated receptor i.e. favoring a Ras domain conformational change—displacement of $\alpha 5$ away from GDP—that weakens interactions between GDP and the Ras domain, allowing GDP to escape

when the G α domains happen to spontaneously separate (top). An activated receptor can also bind to the mutated Gs protein (bottom). (Right) Loss of GDP shifts the equilibrium toward G α conformations with widely separated domains, in absence (top) or in the presence of an activated receptor (bottom). The absence of effects of Tolvaptan (TVP) could be explained by the presence of Gs activated independently from the V2R (top panel).

Panel c: The M255V (or del 68-70) mutations favor the open conformation (unlike the wt Gs) thus shifting the equilibrium toward G α conformations with widely separated domains (bottom) and thus favor receptor-G α protein complex with widely separated domains resulting in higher G protein activity upon loss of GDP (right).

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