Familial neurohypophyseal diabetes insipidus (FNDI) is an autosomal dominant disorder caused by mutations in the arginine vasopressin (AVP) precursor. The pathogenesis of FNDI is proposed to involve mutant protein–induced loss of AVP-producing neurons. We established murine knock-in models of two different naturally occurring human mutations that cause FNDI. A mutation in the AVP signal sequence [A(–1)T] is associated with a relatively mild phenotype or delayed presentation in humans. This mutation caused no apparent phenotype in mice. In contrast, heterozygous mice expressing a mutation that truncates the AVP precursor (C67X) exhibited polyuria and polydipsia by 2 months of age and these features of DI progressively worsened with age. Studies of the paraventricular and supraoptic nuclei revealed induction of the chaperone protein BiP and progressive loss of AVP-producing neurons relative to oxytocin-producing neurons. In addition, 

Avp gene products were not detected in the neuronal projections, suggesting retention of WT and mutant AVP precursors within the cell bodies. In summary, this murine model of FNDI recapitulates many features of the human disorder and demonstrates that expression of the mutant AVP precursor leads to progressive neuronal cell loss.

protein leads to AVP deficiency either by interfering directly with processing of the normal protein or by causing cellular toxicity, or both. In vitro experiments have been used to investigate the processing and cellular effects of transfected AVP mutants (36–39). Mutant AVP precursors are retained in the ER, leading to altered protein processing (36–39) and cell toxicity (36). In addition, when WT and mutant AVP precursors are coexpressed, the mutant protein impairs the trafficking of WT precursors by forming dimers, suggesting a classic dominant negative mechanism (40). These two mechanisms — ER retention and cytotoxicity and formation of mutant/WT complexes — are not mutually exclusive, and together might explain the delayed onset of the disease and the fact that DI occurs despite the presence of a normal allele.

Because studies of pathogenesis are limited in humans, we sought to develop a murine model to analyze the function of AVP-producing neurons in FNDI. We used a targeted gene knock-in (KI) approach rather than transgenic expression of the Avp gene, reasoning that gene dosage and normal regulation of the endogenous gene might be important variables in disease pathogenesis. Two KI models were created, each harboring a different point mutation reported in humans.

The first mutation is the A(–1)T signal peptide mutation, which causes inefficient cleavage of the signal peptide by signal peptidase (10) and a relatively mild phenotype in humans (35). The other mutation is a C67X nonsense mutation that produces an AVP precursor truncated within the NPII domain (15). The expression of this nonsense mutation in cultured cells resulted in lower viability than for cells expressing other types of mutations (36). The heterozygous C67X KI mice develop worsening DI with age and progressive, selective loss of AVP-producing neurons.

Methods

Gene targeting. AVP KI mice were generated by homologous recombination in R1 embryonic stem cells (41). Using a mouse AVP cDNA as a probe, λ phage clones containing mouse Avp and oxytocin (Oxt) genes were isolated from the AFIXII-129Sv genomic library (Stratagene, La Jolla, California, USA). Targeting vectors for the A(–1)T signal peptide mutation and the C67X nonsense mutation were constructed using 7.6- and 12.3-kb genomic DNA fragments, respectively (Figure 1a). The A(–1)T and C67X mutations were introduced by overlapping PCR, creating Scal and NheI restriction sites, respectively: codons TCC(S)/GCC(A) were changed to AGT(S)ACT(T) and codons CGC(R)/TGC(C)/GCC(A) were changed to CGC(R)/TAG(X)/CCC. For selection, a thymidine kinase cassette and a neomycin cassette flanked by loxP sites were introduced. The linearized targeting vector (40 µg) was electroporated into approximately 8 × 10⁶ R1 embryonic stem cells (41), and cells were cultured in the presence of 300 µg/ml G418 (Invitrogen Corp., Gaithersburg, Maryland, USA) and 2 µM ganciclovir (Hoffman–La Roche Inc., Nutley, New Jersey, USA). Homologous recombination was confirmed by both Southern blot and PCR analyses. Seven of 17 clones positive for homologous recombination for the A(–1)T mutation and 17 of more than 100 clones positive for the C67X mutation were then karyotyped to

Figure 1
Targeted mutagenesis of the mouse Avp gene. (a) Targeting strategy. Specific mutations and restriction sites were inserted into exon 1 [A(–1)T; Scal] or exon 2 [C67X; NheI] by homologous recombination. An additional Xbal site (X*) is created after Cre excision of the loxP-Neo-loxP cassette from the A(–1)T targeted allele. Introduced restriction sites were used to detect mutant and WT Avp genes and reverse-transcribed Avp mRNA. White boxes, Avp gene exons; gray boxes, Oxt gene exons. X, Xbal; H, HindIII; E, EcoRI; A, Accl. (b) Southern blot analysis. Xbal- and Scal-digested genomic DNA was hybridized with a 1,214-bp probe (HindIII-EcoRI DNA fragment), labeling a 2,375-bp Xbal-digested DNA for the normal allele and 1,507-bp (XbaI-ScaI) and 404-bp (ScaI-XbaI*) fragments for the A(–1)T mutant allele. Digestion with EcoRI and Scal and hybridization with a 912-bp probe (Xbal-Accl DNA fragment) labeled a 4,578-bp EcoRI-digested DNA for the normal allele and 978-bp (EcoRI-NheI) and 3,600-bp (NheI-EcoRI) DNAs for the C67X mutant allele. (c) RT-PCR analysis for the detection of WT and mutant Avp transcripts in the hypothalamus. A 366-bp cDNA spanning the A(–1)T mutation was amplified using forward and reverse primers located within exon 1 and exon 2, respectively. Restriction digestion with Scal generated a 366-bp band from the normal allele and 265- and 101-bp fragments from the mutant allele. DNA spanning the C67X mutation (267 bp) was amplified by the use of forward (exon 2) and reverse (exon 3) primers. NheI digestion gave rise to 163- and 104-bp fragments derived from the mutant allele.
ensure normal chromosomal composition. Blastocyst injection was performed at the Children’s Memorial Institute for Education and Research transgenic core (Chicago, Illinois, USA) using two embryonic stem cell clones for each of the mutations. All procedures involving animals were approved by the Northwestern University Animal Care and Use Committee.

**Southern blot analysis.** A HindIII-EcoRI DNA fragment (1,214 bp) spanning exon 1 of the mouse Atp gene and an XbaI-Acl fragment (912 bp) spanning exons 2 and 3 were used as probes for screening embryonic stem cells and the progeny of chimeric mice harboring the A(-1)T and C67X mutations, respectively (Figure 1a).

Total genomic DNA purified from tail fragments was digested with XbaI and Scal for the A(-1)T mutant, and with EcoRI and Nhel for the C67X mutant. Digests were separated on a 0.8% agarose gel and transferred with EcoRI and NheI for the C67X mutant. Digests were then run on an 8% polyacrylamide gel. After dry-down, the gel was exposed to a BAS-III imaging plate (Fuji Photo Film Co., Tokyo, Japan) and analyzed with a STORM 860 PhosphorImager and ImageQuant software (Molecular Devices Corp., Sunnyvale, California, USA).

**Immunohistochemistry.** Brains were fixed for 24 hours in 10% neutral buffered formalin at 4°C. Paraffin embedding and sectioning (to 5 μm thickness) was performed by the Pathology Core Facility at Northwestern Memorial Hospital (Chicago, Illinois, USA). Sections were deparaffinized and hydrated with xylenes and a graded series of alcohols, and then washed and permeabilized with TBS/0.025% Tween-20 (Sigma-Aldrich, St. Louis, Missouri, USA). For costaining of NPII and oxytocin, endogenous peroxidase activity was blocked by incubation of slides in 3% hydrogen peroxide/methanol. Sections were blocked with 5% normal horse serum and coincubated with goat anti-NPII and either rabbit anti-oxytocin (anti-OT) antibody (Oncogene Research Products, San Diego, California) or goat anti-rabbit IgG (6 μg/ml, Vector Laboratories Inc.) and alkaline phosphatase–conjugated streptavidin (1:500 dilution, Vector Laboratories Inc.) for 10 minutes each, followed by staining using the Alkaline Phosphatase Substrate Kit III (Vector Laboratories Inc.) for 10 minutes each, followed by staining using the Alkaline Phosphatase Substrate Kit III (Vector Laboratories Inc.). After being washed with TBS/0.025% Tween-20 and blocked with 5% normal goat serum, sections were incubated with rabbit polyclonal anti-OT (anti-oxytocin) antibody (Oncogene Research Products, San Diego, California) for 1 hour and with goat anti-rabbit IgG (6 μg/ml, Vector Laboratories Inc.) and alkaline phosphatase–conjugated streptavidin (1:500 dilution, Vector Laboratories Inc.) for 10 minutes each. Sections were then incubated with biotinylated horse anti-goat IgG (6 μg/ml, Vector Laboratories Inc.) and alkaline phosphatase–conjugated streptavidin (1:500 dilution, Vector Laboratories Inc.) for 10 minutes each, followed by staining using the Alkaline Phosphatase Substrate Kit III (Vector Laboratories Inc.). After being washed with TBS/0.025% Tween-20 and blocked with 5% normal goat serum, sections were incubated with rabbit polyclonal anti-OT (anti-oxytocin) antibody (Oncogene Research Products, San Diego, California) for 1 hour and with goat anti-rabbit IgG (6 μg/ml, Vector Laboratories Inc.) and RTU Horseradish Peroxidase Streptavidin (both from Vector Laboratories Inc.) for 10 minutes each. Sections were then stained with the DAB Substrate-Chromogen System (DAKO Corp., Carpinteria, California, USA). Neurons that were stained in the paraventricular nucleus (PVN) immediately dorsal to the supraopticohypophyseal projection were counted by a blinded observer using light microscopy at ×200 magnification. Only those neurons having a clearly defined cell body and nucleus were counted. Cell counts were expressed as the ratio of the number of NPII-positive neurons to the number of OT-positive neurons.

For costaining of NPII with BiP (Grp78) or Chop (Gadd153), slides were blocked with normal donkey serum and coincubated with goat anti-NPII and either rabbit anti-Gadd153 (F-168, 1:20; Santa Cruz Biotech-
mice produced four times more water than controls (Figure A(–1)T/+: 3 wk: 5.5 ± 3). Mice consumed significantly more water over the course of 24 hours than did WT and C67X lines. The normal and mutant alleles were expressed at similar levels in both mouse lines.

**Results**

**Generation of Avp KI mice.** Mice harboring mutations in the Avp gene were created by homologous recombination using standard techniques and the targeting constructs illustrated in Figure 1a. Chimeric males were bred to WT 129SvJ females and germline transmission was detected by PCR and restriction digestion (data not shown). Animals heterozygous for the mutation and containing the neomycin cassette were bred to transgenic 129SvJ animals expressing Cre recombinase under the control of the cytomegalovirus promoter (42), and excision of the neomycin cassette was confirmed by PCR using primers flanking the loxP site (data not shown). For one of two founder lines for each mutation, correct targeting was confirmed by Southern blot analysis (Figure 1b). For the A(–1)T mutation, the mutant allele introduced a new XbaI site and excision of the neomycin cassette yielded a 267-bp fragment. Subsequent breeding yielded animals containing the mutations but lacking the Cre recombinase. These animals and their litter-matched controls were used in all subsequent studies.

**Expression of the mutant Avp genes in the mouse hypothalamus.** RT-PCR was performed to assess expression of the Avp transcripts from normal and mutant alleles in the mouse hypothalamus (Figure 1c). RT-PCR yielded amplification products of 366 and 267 bp for the A(–1)T and C67X lines, respectively. The A(–1)T and C67X normal and mutant alleles could be distinguished in heterozygotes by digesting the amplification products with Scal and NheI, respectively. Scal digestion of the PCR products from AvpA(–1)T/+ mice produced a 366-bp band from the normal allele and 265- and 101-bp bands from the mutant allele. NheI digestion of PCR products in AvpC67X/+ mice yielded a 267-bp WT band and 163-bp and 104-bp fragments from the mutant allele. The normal and mutant alleles were expressed at similar levels in both mouse lines.

**AvpC67X/C67X** mice die soon after birth, whereas **AvpA(–1)T/A(–1)T** mice are viable. The mutant mice were bred to homozygosity using standard heterozygote crosses. For the A(–1)T mutation, progeny with the expected mendelian ratio of genotypes were obtained. In contrast, crosses of AvpC67X/+ mice produced no homozygotes at weaning age (i.e., 3 weeks) from 12 litters. As an abnormal number of dead 2- to 4-day-old pups were observed in the cages with the live offspring from C67X heterozygote crosses, 1-day-old pups from 11 additional AvpC67X/+ crosses were genotyped. These litters contained 17 WT, 33 heterozygote, and 15 homozygote pups, demonstrating that C67X homozygotes are born at the expected frequency but die soon after birth, likely from dehydration. These data are summarized in Table 1 and Table 2.

**AvpC67X/+ mice, but not AvpA(–1)T/+ or AvpA(–1)T/A(–1)T mice, display altered water intake, urine output, and urine osmolality.** Water intake, urine output, and urine osmolality of mutant mice and litter-matched WT controls were measured to determine whether these mutations caused altered water balance in mice similar to effects seen in humans. No significant differences were observed between homozygous AvpA(–1)T/A(–1)T mice, heterozygous AvpA(–1)T/+ mice, and controls at 12 months of age (Figure 2). In the case of the C67X mutation, 6-month-old AvpC67X/+ mice consumed significantly more water over a 24-hour period than did WT Avp+/+ controls (Figure 3a). Beginning at 1 month of age, heterozygous AvpC67X/+ mice also produced more urine over the course of 24 hours than did their litter-matched Avp+/+ controls; this pathology became progressively worse with age (Figure 3b). Two-month-old AvpC67X/+ mice produced four times as much urine as controls, and by age 6 months the difference was nearly tenfold. Conversely, urine osmolality was decreased in 2-month-old AvpC67X/+ mice and

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Data are mean ± SEM.

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6-month-old AvpC67X/+ mice compared with controls (Figure 3c). Thus, like humans with FNDI, AvpC67X/+ mice produce excessive amounts of dilute urine but compensate by increasing water intake.

AvpC67X/+ mice exhibit decreased serum AVP concentrations. Serum AVP concentrations were measured at various ages using a specific ELISA to determine whether the metabolic abnormalities seen in the AvpC67X/+ mice could be caused by a reduction in circulating AVP. Three-, 6-, 9-, and 12-month-old AvpC67X/+ mice had lower serum AVP concentrations than did their WT littermates (Figure 4a). Serum AVP concentrations were also measured in 1-month-old mice after a 24-hour period of water deprivation to study the changes in AVP secretion in response to this strong stimulus for AVP secretion. Following water deprivation, the concentration of serum AVP in AvpC67X/+ mice was only 20% of the level in Avp+/+ mice (Figure 4b).

Immunohistological detection of vasopressinergic neurons in the hypothalamus. Immunohistochemical analysis was performed to determine whether there was loss of AVP neurons in the AvpC67X/+ mice. Hypothalami of 1-, 2-, 6-, and 18-month-old WT and mutant mice were immunostained with polyclonal antibodies against NPII and OT. There was no significant difference between the two groups in the numbers of oxytocinergic neurons in the PVN (Figure 5a, brown stain). In contrast, AvpC67X/+ mice exhibited fewer vasopressinergic neurons than Avp+/+ mice beginning at 2 months of age, with a near-total loss of AVP-expressing neurons by 18 months (Figure 5a, blue stain). Neurons were counted and expressed as a ratio of vasopressinergic to oxytocinergic neurons as a way to normalize between sections. The ratio of vasopressinergic neurons to oxytocinergic neurons in AvpC67X/+ mice was statistically lower than in Avp+/+ mice at 2, 6, and 18 months of age (PVN, Figure 5b). A similar progressive loss of vasopressinergic neurons was seen in the supraoptic nucleus (Figure 5b).

In the PVN of Avp+/+ mice, both the cell bodies and a dense network of neuronal projections stained for NPII (Figure 5a). In contrast, staining of the projections was diminished in the AvpC67X/+ mice at all ages studied (compare upper and lower panels in Figure 5a). Figure 6 illustrates the entire tract from the PVN to the supraoptic nucleus in young mice. Note that NPII immunostaining in the neuronal projections seen in the Avp+/+ mice is nearly absent in the AvpC67X/+ mice at 1 month after birth, preceding the overt loss of vasopressinergic neuronal cell bodies.

ER stress–associated protein expression in vasopressinergic neurons. Levels of the molecular chaperone BiP (Grp78) and an ER stress–induced transcription factor, Chop (Gadd153), were measured in the PVN by immunohistochemistry. Chop was not detected in either Avp+/+ or AvpC67X/+ mice (Figure 7a). BiP was also absent in Avp+/+ mice, but was strongly induced in AvpC67X/+ mice (Figure 7b). Costaining for NPII confirms that BiP-expressing cells are vasopressinergic.

Discussion
In this study, we generated a murine model of FNDI that faithfully replicates several features of the human

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Data are mean ± SEM. N/A, not applicable.
disorder, including autosomal dominant transmission, delayed onset and progressive worsening of the defect in water retention, and loss of AVP-producing neurons in the hypothalamus.

The delayed onset of FNDI has led several groups to speculate that the mutant AVP precursor causes cellular toxicity, leading to eventual loss of AVP-producing neurons (reviewed in ref. 35). This hypothesis is supported by in vitro studies showing accumulation of mutant vasopressin precursors in transfected cell lines (36–38, 40, 43) and cytotoxicity of certain mutants (36). Of note, there is little evidence of apoptosis, suggesting that cell death may occur via other pathways (36).

The processing of mutant vasopressin has also been examined using metabolic labeling studies (37, 38, 40, 43). These experiments document persistent endoglycosidase H sensitivity, consistent with prohormone retention in the ER. The ER becomes distended (43), and immunoreactive protein forms perinuclear punctate complexes rather than diffuse cytoplasmic staining (36). Coexpression of WT and mutant AVP precursors results in the formation of WT/mutant dimers, and the mutant prohormone delays WT protein processing (40). Thus, it appears that the mutant vasopressin precursor may exert at least two distinct cellular effects: (a) formation of complexes with the WT precursor protein that impair its processing, and (b) accumulation of misfolded proteins in the ER, causing cellular toxicity by mechanisms that remain to be fully characterized. Because these in vitro experiments involve high-level expression of the mutant protein and occur in dividing cell lines, it is desirable to develop models that more closely mimic conditions in vivo.

Two animal models have been used previously to study central DI. In the Brattleboro rat, a naturally occurring single base deletion in the NPII coding sequence produces AVP precursors with continued translation into the mRNA poly-A tail, leading to a polylysine stretch of amino acids. In these animals, DI is transmitted in an autosomal recessive manner (44), suggesting that it is caused by an absolute deficiency of AVP. Although mutant precursors are retained within the ER, there is no apparent loss of AVP-producing neurons (45). The absence of cell death in the Brattleboro rat might be explained by differences in the effects of various mutant proteins or by species differences in the cellular mechanisms for coping with mutant AVP precursors.

Of interest, the same mutant described here (C67X) has been expressed in transgenic rats (46, 47). In this case, features of DI were manifest only as a small increase in water intake after repeated dehydration (46). However, there was evidence of retention of mutant precursors.

**Figure 3**

Osmoregulation in mice with the C67X mutation. (a) Water intake of mice over a 24-hour period. Six-month-old *Avp*<sup>C67X/+</sup> mice drank significantly more water than did their WT littermates. (b) Urine output of mice over a 24-hour period. One-, 2-, and 6-month-old *Avp*<sup>C67X/+</sup> mice excreted significantly more urine than did their WT littermates. (c) Urine osmolality. Urine samples from *Avp*<sup>C67X/+</sup> mice at 2 and 6 months of age were more dilute than those of their WT littermates. n ≥ 5 for all groups. Data are mean ± SEM. *P < 0.05.

**Figure 4**

Serum AVP analysis. (a) Serum AVP levels of *Avp*<sup>C67X/+</sup> and *Avp*<sup>+/+</sup> mice at five different ages were quantified using ELISA. At 3, 6, 9, and 12 months of age, serum from *Avp*<sup>C67X/+</sup> mice contained significantly less immunoreactive AVP than did serum from the WT mice (n ≥ 4 for all groups). (b) Serum immunoreactive AVP concentrations from 1-month-old *Avp*<sup>C67X/+</sup> and WT mice following a 24-hour period of water deprivation. Values for *Avp*<sup>C67X/+</sup> samples were significantly lower than those for WT samples. Data are mean ± SEM. *P < 0.05.
in distended ER. In addition, vesicles contained markers of the lysosomal degradation pathway, suggesting autophagy (47). There was no evidence of cell death in the transgenic rats. Because the same mutant was expressed in this transgenic model and in our KI mice, it is interesting that the mice exhibit features more typical of the human disorder. Although this may be due to species differences between rats and mice, differences in gene dosage also provide a plausible explanation. The transgenic rats retain two normal vasopressin alleles in addition to the transgenic mutant gene, whereas the KI mice possess one normal vasopressin allele and one mutant allele, analogous to the human condition. Our RT-PCR studies document similar expression levels of the WT and mutant alleles, consistent with the fact that AVP expression is driven by the endogenous gene, which remains in its normal chromosomal locus. In view of evidence for interaction of WT and mutant precursor proteins during processing (40), gene dosage could affect the ratio of mutant-WT complexes. There may also be differences in the physiologic stimuli for vasopressin expression. For example, chronic mild dehydration in the mice would lead to upregulation of vasopressin gene expression, 50% of which is comprised of mutant precursors. Based on in vitro metabolic studies, processing of the mutant precursor is delayed relative to processing of the WT protein (36). Thus, a relative excess of mutant protein is predicted to accumulate over time.

The phenotypes of the mice with two different FNDI mutations were distinct. In the case of the signal sequence A(–1)T KI mutant, there was no apparent DI

**Figure 5**
Number of vasopressinergic neurons in the PVN and supraoptic nuclei (SON) of the hypothalamus decrease with age in Avp<sup>C67X/+</sup> mice. (a) Representative hypothalamic sections from the PVN of 1-, 2-, 6-, and 18-month-old Avp<sup>C67X/+</sup> mice (bottom row) and their WT littermates (top row) immunostained for NPII (blue, alkaline phosphatase) and OT (brown, DAB) are shown. Although the PVN of 1-month-old Avp<sup>C67X/+</sup> mice and WT mice contained approximately equal numbers of vasopressinergic cell bodies (left panels), the PVN of the 18-month-old Avp<sup>C67X/+</sup> mouse appears to have only two AVP-producing neurons (arrows, lower right panel). The number of oxytocinergic neurons is unchanged across all ages and genetic variations. Magnification, ×200. (b and c) Ratios of the numbers of magnocellular vasopressinergic neurons to oxytocinergic neurons in the PVN (b) and supraoptic nucleus (SON) (c). Sections from 2-, 6-, and 18-month-old Avp<sup>C67X/+</sup> mice showed significantly lower ratios than their littermates (n = 6 animals for each group; three sections were counted for each animal). Data are mean ± SEM. *P < 0.05.

**Figure 6**
Intracellular localization of the Avp gene products immunoreactive for NPII. In WT animals at both 1 and 2 months of age, immunoreactive NPII (blue, alkaline phosphatase) was present in the neural projections between the hypothalamic PVN and supraoptic nucleus (upper panels). In contrast, staining of immunoreactive NPII is confined to the cell bodies of the 1- and 2-month-old Avp<sup>C67X/+</sup> mice (lower panels). Arrows in the inset image in the upper left panel highlight the neural projections in 1-month old animals; the inset in the lower left panel shows an absence of staining outside the cell bodies. This is further illustrated in the insets in the right panels, which contain confocal micrographs from 2-month-old animals. Magnification, ×100; insets, ×400.
phenotype and we did not detect loss of AVP-producing neurons, even in homozygous mice. This mutation gives rise to aberrant preproAVP that is glycosylated but retains the signal peptide as a result of inefficient cleavage. Consistent with the in vivo results, cell lines expressing the A(−1)T mutant were more viable than cells expressing the C67X mutant (36). In contrast with the A(−1)T mutant, a severe and consistent phenotype was seen in mice carrying the C67X mutation. The more severe phenotype in the C67X mutant is consistent with in vitro studies that show it is the most cytotoxic among several AVP mutants studied (36). The C67X mutation was lethal in the homozygous state, presumably due to a complete lack of functional AVP. Heterozygotes were more informative, however. Water intake and urine volumes were increased concomitant with decreased urine osmolality and serum AVP. By 6 months of age, the 24-hour urine volume of the C67X mice was about one-third of their body weight. This is not dissimilar from affected children, who may produce several liters of urine each day and must be provided with easy access to fluids to avoid severe dehydration. Like humans, the mice are able to compensate for profound diuresis by drinking copious amounts of water, indicating that their thirst mechanism remains intact.

One of the advantages of developing an animal model of FNDI is the ability to correlate the phenotype with pathological changes in the brain regions that produce AVP. In C67X heterozygotes, the number of AVP-expressing neurons in the PVN of the hypothalamus decreased beginning at 2 months of age. Comparison with OT-producing cells demonstrated that the neuronal cell loss was specific for AVP-producing neurons. The progressive loss of AVP-producing neurons correlated with the worsening features of DI, suggesting gradual cell loss over time.

Apoptosis was not detected using a TUNEL assay or immunohistochemical analyses of apoptosis markers (caspase-3, cathepsin D; data not shown). However, given the small number of vasopressin-producing neurons and the progressive loss of cells over weeks to months, these assays are unlikely to be sensitive enough to detect apoptosis of a small number of neurons. There was no apparent induction of the ER stress protein Chop (48), which has been reported to be proapoptotic (49, 50). In contrast, there was marked induction of BiP, a member of the HSP70 family of molecular chaperones. BiP binds avidly to misfolded proteins whose transport from the ER is blocked, and BiP expression is increased as part of the “unfolded protein response” (reviewed in ref. 51). Thus, elevated levels of BiP are consistent with the retention of mutant AVP precursors in the ER of C67X mice. It is notable that NPII immunoreactivity was confined to the cell bodies of magnocellular neurons. The absence of staining in the neuronal projections suggests that AVP precursors produced from the normal allele may also be retained within the ER through interaction with mutant precursors (40).

Thus, the vasopressinergic neurons of C67X mice exhibit ER accumulation of vasopressin precursors and induction of the BiP chaperone response. These neurons are selectively lost over several months, presumably the consequence of toxic effects of the mutant proteins. However, the ultimate steps that lead to cell loss remain unknown.

We propose that FNDI can be added to a list of neurodegenerative disorders that includes Alzheimer disease, Parkinson disease, and various CAG-repeat diseases (52–54). In the case of FNDI, the combination of in vitro and in vivo models suggests cellular dysfunction by several different mechanisms, including dominant...
negative activity by interactions of mutant and WT precursors, accumulation of mutant precursors in the ER leading to stress protein responses and autophagy, and cellular toxicity by pathways that remain incompletely defined. Ultimately, the loss of AVP-producing neurons depletes AVP production below a level where compensatory responses can prevent DI. Because the C67X KI mouse faithfully replicates many features of FNDI, it provides a useful model for studying the specific molecular and cellular mechanisms responsible for the pathology seen in FNDI, with potential implications for other neurodegenerative diseases.

Acknowledgments

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