Evidence of hypothalamic mitochondrial dysfunction and neurodegeneration in the anorectic *anx/anx* mouse

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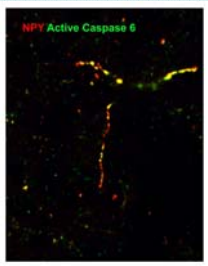

This study provides evidence of mitochondrial dysfunction and selective degeneration of food intake regulating neurons in the arcuate nucleus (Arc) of the hypothalamus in the anorectic *anx/anx* mouse. We show that the *anx/anx* mouse displays lower levels of the OXPHOS complex I (CI) assembly factor *Ndufaf1,* accompanied with lower levels of fully assembled CI and reduced CI activity. We also demonstrate increased levels of reactive oxygen species (ROS) and superoxide dismutase 2 (SOD2) in the *anx/anx* hypothalamus, indicating oxidative stress, commonly seen in CI deficiency. We hypothesize that the increased oxidative stress results in the degeneration of Arc neurons seen in these mice.

Figure 1. NPY-immunoreactive (red) axons in hypothalamus of an *anx/anx* mouse. Green color indicates expression of activated caspase 6, a marker of axonal degeneration. Note the co-expression (yellow) of NPY and caspase 6, indicating axonal degeneration of the NPY-neurons.

Figure 2. A 17 days old *anx/anx* mouse (left) and a +/+ litterrmate (right**).**

The anorectic *anx/anx* mouse

The *anx/anx* mouse is an attractive model for studying disturbed feeding behavior. This mouse arose by a spontaneous mutation (anorexia, allele symbol *anx*), and it is characterized by low food intake and premature death, likely due to the severe starvation, around 3 weeks after birth. The *anx/anx* mouse also exhibits a number of neurological abnormalities such as body tremors, head weaving, hyperactivity and uncoordinated gait.

Several neurotransmitter and neuropeptidergic systems involved in the regulation of food intake and energy metabolism, have been reported to be disturbed in the *anx/anx* mouse. The majority of these aberrances are centered around the hypothalamus, where a neuronal network important for the control of food intake originates.

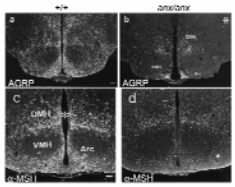

Figure 3. Immuno-histochemical stainings showing aberrant appearance of the food intake regulating neuro-peptides AGRP and α-MSH in hypothalamus of the the *anx/anx* mouse at P21.

Interestingly, these aberrances overlap in both time and space with activation of microglia, indicating inflammatory and/or degenerative processes.

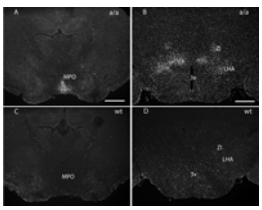

Figure 4. Micrograph of Iba1 immunofluorescence in *anx/anx* mice (upper panel) demonstrating increased number of activated microglia in several hypothalamic regions.

Hypothalamic MHC class I expression

Region specific expression of MHC class I by both microglia and neurons (only in Arc) provides further proof for inflammatory and/or degenerative processes in the *anx/anx* hypothalamus.

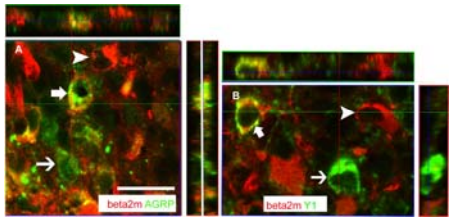

Figure 5. Double labeling (thick arrows) of MHC class I related protein β2m (red) and AGRP (green in A) or Y1 (green in B) in *anx/anx* Arc.

Apoptotic/degenerative signs in *anx/anx* hypothalamus

Expression of activated Caspase 6, a marker for axonal degeneration, in AGRP/NPY neurons (Fig. 1) and increased number of apoptotic cells in *anx/anx* hypothalamus at P21 strongly indicate hypothalamic degeneration in the *anx/anx* mouse.

The *anx/anx* mouse show CI deficiency

Affymetrix microarray analysis, followed by Ingenuity pathway analysis (IPA), of Arc, identified several genes with altered expression, related to oxidative stress and mitochondrial functions, in the *anx/anx* mouse. The microarray result, in combination with the phenotypic resemblance with CI-deficiencies, indicate that the starvation and neurodegeneration observed in the *anx/anx* mouse are related to mitochondrial defects.

Additional evidence for CI deficiency were provided by high resolution respirometry assessments, that showed reduced CI activity in *anx/anx* hypothalamus. Furthermore, native western blot demonstrated decreased levels of fully assembled CI and an accumulation of possible sub-complexes in the *anx/anx* hypothalamus.

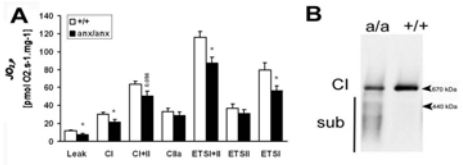

Figure 6. Mitochondrial respiration in hypothalamus samples (A) and Native western blot of complex I (B).

Signs of oxidative stress in *anx/anx* hypothalamus

Complex I deficiency is often associated with increased levels of ROS. Accordingly, we show increased levels of ROS and SOD2 (a ROS scavenger) in the hypothalamus of the *anx/anx* mouse. These results indicate hypothalamic oxidative stress, possibly leading to the observed neurodegeneration.

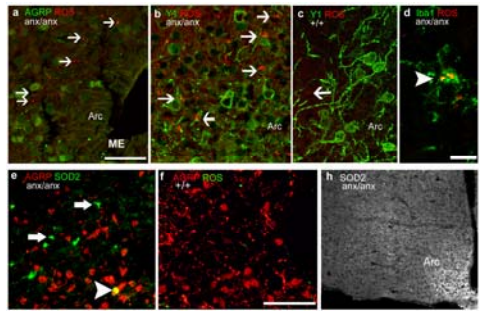

Figure 7. Micrographs showing ROS (red) staining and immunofluorescent labeling for AGRP (green) (a), Y1 (green) (b,c), Iba1 (green) (d) in Arc of *anx/anx* (a,b,d) and +/+ (c) mice at P21. Immunofluorescence micrographs showing SOD2 (green in e, f) (e, f, g) and AGRP (red) (e, f) in Arc of an *anx/anx* mouse (e, g) and +/+ mouse (f) at P21.

Ndufaf1 – the *anx*-gene?

Using positional cloning we have mapped the *anx* gene to a 0.2cM interval on mouse Chromosome 2. This interval contain the *Ndufaf1* gene, encoding a CI assembly factor, that was shown to be 2-fold down regulated. In addition, allele-specific expression analysis, using a silent C/T SNP, showed that the down-regulation of *Ndufaf1* is specific to the *anx* allele, rather than a secondary effect of the phenotype. However, mutation analysis of *Ndufaf1* coding sequence has not revealed any *anx*-specific alterations. Thus, should *Ndufaf1* be equivalent to the *anx*-gene this would indicate that the *anx* mutation lies within regulatory sequences.

Conclusion

Taken together, the results from this study support the hypothesis of mitochondrial dysfunction and selective degeneration of food intake regulating arcuate neurons in the *anx/anx* mouse.

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The *anx/anx*–team. From left: Charlotte Lindfors (PhD-student), Ida Nilsson (postdoc) and Jeanette Johansen (PhD and team-leader)

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