News Release

Title

Endoplasmic reticulum chaperone BiP/GRP78 knockdown leads to autophagy and cell death of arginine vasopressin neurons in mice

Key Points

- **BiP is a pivotal endoplasmic reticulum (ER) chaperone which modulates protein folding and degradation of aberrant proteins.**
- **BiP knockdown in AVP neurons led to autophagy together with ER stress and cell death of AVP neurons.**
- **Autophagic inhibition exacerbated AVP neuronal loss due to BiP knockdown in AVP neurons, indicating a protective role of autophagy in AVP neurons under ER stress conditions.**

Summary

The immunoglobulin heavy chain binding protein (BiP), also referred to as 78-kDa glucose-regulated protein (GRP78), is a pivotal endoplasmic reticulum (ER) chaperone which modulates the unfolded protein response under ER stress. Our previous studies showed that BiP is expressed in arginine vasopressin (AVP) neurons under non-stress conditions and that BiP expression is upregulated in proportion to the increased AVP expression under dehydration. To clarify the role of BiP in AVP neurons, we used a viral approach in combination with shRNA interference for BiP knockdown in mouse AVP neurons. Injection of a recombinant adeno-associated virus equipped with a mouse AVP promoter and BiP shRNA cassette provided specific BiP knockdown in AVP neurons of the supraoptic (SON) and paraventricular nuclei (PVN) in mice. AVP neuron-specific BiP knockdown led to ER stress and AVP neuronal loss in the SON and PVN, resulting in increased urine volume due to lack of AVP secretion. Immunoelectron microscopy of AVP neurons revealed that autophagy was activated through the process of AVP neuronal loss, whereas no obvious features characteristic of apoptosis were observed. Pharmacological inhibition of autophagy by chloroquine exacerbated the AVP neuronal loss due to BiP knockdown, indicating a protective role of autophagy in AVP neurons under ER stress. In summary, our results demonstrate that BiP is essential for the AVP neuron system.

Research Background

The ER plays an essential role in synthesis, folding, assembly and transport of secretory and transmembrane proteins. Disturbance of ER homeostasis causes the accumulation of misfolded proteins in the ER lumen leading to ER stress. BiP/GRP78, is one of the most abundant ER chaperones. BiP binds to newly synthesized polypeptides to promote their folding and binds to

misfolded proteins to facilitate correct refolding and prevent their aggregation.

AVP, an antidiuretic hormone that promotes water reabsorption in the kidney, is mainly synthesized in the magnocellular neurons of SON and PVN in the hypothalamus. AVP mRNA expression in the SON and PVN is relatively high, and AVP synthesis and release are upregulated by only a 1-2% increase in plasma osmolality, suggesting that AVP neurons must meet a large demand for AVP production as specialized secretory cells. AVP precursors are subjected to proper folding in the ER, and through the folding process, some degree of AVP precursors undergo ER-associated degradation. Furthermore, knockout of the Sel1L-Hrd1 protein complex, a principal ER-resident E3 ligase in mammalian ERAD, is reported to cause marked retention and aggregation of AVP precursors in the ER, resulting in polyuria due to AVP deficiency. These data indicate that ER protein quality control is essential for appropriate AVP synthesis and release.

Our previous studies showed that BiP is expressed in AVP neurons under non-stress conditions and that its expression is upregulated in proportion to the increase in AVP expression under dehydration. While these data suggest that BiP might be involved in the synthesis and release of AVP, the role of BiP in the AVP neuron system has not been fully clarified. In the present study, we specifically ablated BiP expression in AVP neurons by utilizing virally-mediated shRNA interference and analyzed the morphology of AVP neurons as well as fluid homeostasis in mice.

Research Results

In the current study, we ablated BiP exclusively in AVP neurons by injection of recombinant adeno-associated virus vectors expressing BiP shRNA into the SON and PVN. AVP neuron-specific BiP knockdown led to ER stress and cell death of AVP neurons. Electron microscopic analyses revealed that autophagy was induced in AVP neurons through the process of AVP neuronal loss by BiP knockdown. Autophagic inhibition by a lysosomal inhibitor chloroquine exacerbated AVP neuronal loss due to BiP knockdown in AVP neurons.

Research Summary and Future Perspective

Our data demonstrate that BiP knockdown in AVP neurons leads to ER stress and activates autophagy in AVP neurons followed by AVP neuronal loss, suggesting that BiP is essential for the AVP neuron system. In addition, autophagic inhibition exacerbates AVP neuronal loss due to BiP knockdown in AVP neurons, indicating a protective role of autophagy in AVP neurons under conditions of ER stress.

Publication

Endoplasmic reticulum chaperone BiP/GRP78 knockdown leads to autophagy and cell death of arginine vasopressin neurons in mice. **Scientific Reports**.

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