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Chronic stress and the adult zebrafish brain: the importance of the GABA signaling system by a gene-expression perspective

1. Introduction

Animals live in an environment that poses fundamental surviving challenges, which need to be sensed and interpreted to produce proper behavioral responses. In the vertebrate brain, potentially life-threatening conditions cause the activation of defensive survival circuits.[1] Those involve descending crosstalks between the cortex and the amygdala, and the latter and the hypothalamus, as well as ascending projections traveling the opposite direction.[2-7] Descending brainstem projections affect the activity of hypothalamic nuclei, too.[4-6] The ultimate effect of the activation of defensive survival circuits is a switch-on of the endocrine stress response, achieved through communications between the hypothalamus and the hypophysis.[1,4,5] This starts with the release of corticotropin-releasing hormone (CRH) by CRH cells located in the paraventricular nucleus (PVN) of the hypothalamus. CRH in turn stimulates the secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary, which subsequently stimulates the synthesis and systemic secretion of glucocorticoids from the adrenal cortex into the bloodstream[4,5]. Glucocorticoids (mainly cortisol) regulate the energy status of the body, promoting energy mobilization and therefore facilitating a reaction to a threat.[6] The physiological pathway, starting from the nervous system and eventually affecting the adrenal cortex, is termed hypothalamo-pituitary-adrenal (HPA) axis in mammals.[6] In teleosts, no condensed adrenal gland is present; it is rather possible to identify sparse cell clusters analogous either to the adrenal medulla or cortex.[8] Thus, for fish the definition hypothalamo-pituitary-interrenal (HPI) axis is used.

In the adult vertebrate brain, inhibition of defensive survival circuits is mediated by the major inhibitory neurotransmitter γ-aminobutyric acid (GABA), which is synthesized from α, L-glutamate by glutamate decarboxylases (GADs).[9,10] GABA is widely employed as signaling molecule in defensive survival circuits, as reported by immunological studies on GADs distribution in both mammals and fish.[10-13] The use of GABA is also witnessed by $GABA_A$ receptor subunits, which assemble in a region-specific fashion with a strict stoichiometry.[14-19] $GABA_A$ receptors are homo- or heteropentamers; in mammals there are nineteen genes encoding for its monomers, in zebrafish at least twenty-three.[20,21] In the amygdala of rats and mice α_2 and α_3 are detected to the highest level among the α monomers.[14,17-19] The β_3 and γ_1 subunits are also produced to high levels; in this brain area $\alpha_2\beta_3\gamma_1$, $\alpha_3\beta_3\gamma_1$ $GABA_A$ receptors are found. The γ_2 monomer is present as well, and receptors incorporating this subunits may also be located in the amygdala.[14,19] In the hippocampus $\alpha_5\beta_3\gamma_2$ $GABA_A$ channels are present.[14,15,19,22] The pattern of $GABA_A$ receptor subunits expression is somewhat different between rats and mice in the hypothalamus. In the former species the $\alpha_2\beta_3\gamma_1$ combination is detected, whereas the latter also presents the α_2, β_1, and γ_2 monomers detected to a certain level.[14,19] Chronic stress impairs GABA signaling in

the defensive survival circuits, both at the gene expression level[23,24] and in terms of altered chloride permeability of GABA target cells.[25]

Also the HPA-axis is under GABAergic control. CRH neurons in the PVN receive tonic inhibitory GABAergic input that constrains CRH neuronal activity under basal conditions.[26] Following an acute threat, the frequency of spontaneous inhibitory postsynaptic currents is reduced up to 5 h later, causing an increase in circulating glucocorticoid levels. Chronic stress and sustained increases in systemic glucocorticoid levels lead to suppression of GABAergic inhibition of CRH neurons, by a reduction in GABA synapses.[26] Also the composition of the $GABA_A$ receptors in the PVN can change in response to chronic stress, towards increased expression of neurosteroid-sensitive $GABA_A$ receptor δ subunit-containing receptors.[24] Stress-derived steroid hormones can be metabolized to neurosteroids (such as THDOC and allopregnanolone), which can act as positive allosteric modulators of $GABA_A$ receptor δ subunit-containing receptors, potentiating the tonic component of GABAergic inhibition.[27] This would provide a reciprocal regulation of stress hormones and GABA receptors, where GABAergic inhibition regulates the HPA-axis and the production of stress hormones and derived neurosteroids also alter this GABAergic inhibition during chronic stress.[27]

2. Aim of the study in detail

The aim of the project is to investigate the effect of acute versus chronic stress on the GABA signaling system in the adult zebrafish brain. Our group has recently characterized the GABA signaling system in the adult zebrafish brain[21], and qPCR primers have been developed to quantify mRNA expression of $GABA_A$ receptor subunits and GADs in specific brain areas. Both female and male zebrafish will be studied, allowing for investigation of potential sex differences.

3. Techniques

a. Dissection of the zebrafish brain;
b. RNA extraction and RNA quality evaluation;
c. Reverse transcription of RNA into cDNA;
d. RT-qPCR;
e. Measurement of water total cortisol;
f. Measurement of plasma cortisol;
g. Data analysis.

4. Supervisors

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