Understanding the contribution of stress to generation of auditory pathway disorders

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Abstract

Chronic stress has been implicated in the phantom sound of tinnitus, a ringing, hissing or buzzing sensation. Tinnitus, a sometimes debilitating condition, is hypothesized to be caused by the loss of GABA (g-aminobutyric acid)-mediated inhibition leading to spontaneous hyperactivity within the central auditory system. Since tinnitus is often linked to chronic stress, we hypothesized that chronic stress would reduce the expression of GAD67, which is involved in synthesizing GABA, in the auditory cortex. We used immunohistochemistry and Western blot to evaluate the expression of GAD67 in the auditory cortex of 5 control rats and 5 chronically stressed rats. We compared weight gain for control and experimental groups to confirm chronic restraint was inducing stress. Western blot analysis showed that GAD67 expression was significantly reduced in the auditory cortex of chronically stressed rats. Immunolabeling studies are currently underway to localize GAD67 and anti-mouse as the primary and secondary antibodies, respectively. Diaminobenzidine (DAB) reactions along with hydrogen peroxide were used to visualize the staining.

Introduction

Figure 1a – Represents the auditory pathway from the ears to the auditory cortex. 1b – Shows the pathway of the signal in a neuron.

GABA expressing neurons are present in relatively ample amounts, approximately 20-25% of the total population of neurons (Burinova et al. 2009).

GABA has been quantified by using immunohistochemistry and western blotting techniques and analysis on GAD65 and GAD67, key types of glutamate decarboxylase that catalyze the synthesis of GABA in the AC and the IC.

Disruption in the balance of excitation and inhibition can lead to a disruption in responses received in the higher auditory pathways, described as an alteration in the function of the fine tuning mechanism.

Hypothesis

Stress can further elevate the severity of the dysfunction of tinnitus or hyperacusis by inducing changes in the expression of glutamate decarboxylase (GAD), which then leads to a decrease in the production of GABA.

Methods

Subjects:

Adult male Sprague Dawley rats (2-3 months old).

Chronic Stress:

A reputable paradigm using plastic restraint devices was used as a model of chronic stress in rodents (6 hours per day for 21 consecutive days) to induce changes in brain structure and function.

Immunostaining:

The rat brain sections were used for visualizing the expression of GAD67. This was done using the standard procedure for immunostaining, employing GAD67 and anti-mouse as the primary and secondary antibodies, respectively. Diaminobenzidine (DAB) reactions along with hydrogen peroxide were used to visualize the staining.

Results

Figure 2a - Shows all of the brain sections that can be taken from a rat. 2b - Shows the binding techniques of the primary and secondary antibodies to procure specific staining.

Taking Images (Layer by Layer):

As we took microscope images we used a layer by layer basis to quantify the amount of staining present on each one. This is because certain layers contain a lot more GABA expressing neurons than others.

Results Cont’d

GAD67 immunoreactivity neurons in auditory cortex

Figure 3: Using Imagi to take pictures of the slides at (a) 2.5x and (b) 10x. Take images on the auditory cortex in a layer formation of 1-6.

Layer 6
Layer 5
Layer 4
Layer 3
Layer 2
Layer 1

Figure 4: The chronically stressed rats (stressed 6 hours/day for 21 days, n=5) had gained significantly less body weight than the controls. This part of the experiment was used to verify that this is an effective model of stress in a rodent. Weight loss of a stressed animal is one among several determinants of stress in their environment.

Western Blot:

Figure 5: The chronically stressed rats (stressed 6 hours/day for 21 days, n=5) had significantly less expression of GAD67 in the auditory cortex. Tissue was homogenized from fresh brains.

Figure 6: First column in the figure shows superficial layers and second column shows deep layers of auditory cortex. Visual inspection suggest that neurons in deep layers of stressed animals showed a slight decrease in GAD67 immunoreaction compared to control.

Figure 7: Image J quantification of gad 67 immunoreactions showed slight decrease in optical density analysis in layer 6 of auditory cortex (deep layer), but a slight increase in layers 3 and 4 (superficial) of stressed animal compared to controls. Changes in all layers, however, were not significant enough to verify hypothesis.

Conclusion

1. The model for chronic restraint stress was effective at creating a rodent stress model. The restraint condition resulted in less weight gain by those rats.
2. Chronic stress significantly downregulated GAD67 protein expression in auditory cortex as verified by Western Blot analysis. These results suggested that chronic stress disrupted GABA-mediated inhibition.
3. To confirm the Western Blot results, immunolabeling studies of GAD67 were performed on auditory cortex. The GAD67 immunolabeling results failed to identify a significant decline in GAD67 in any layers of auditory cortex. Thus, the immunolabeling data did not support the Western Blot analysis for reasons that remain unclear.

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