The Role Of GABAergic Neurotransmission In The Development Of Locomotive Behavior

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Abstract

Zebrafish provide an excellent exemplar system to study the development of the vertebrate nervous system, as embryos develop outside of the mother and are transparent. Zebrafish embryos demonstrate three stages of locomotive behavior through development: first spontaneous movement, then touch evoked tail cells, and finally swimming. In this study, we attempt to elucidate the role that GABAergic neurotransmission plays during vertebrate locomotive behavior development using two approaches. In the first approach, we aim to determine stage of locomotive behavior development that requires GABAergic neurotransmission. Pharmacological drugs that either stimulate or antagonize GABA receptors were injected into zebrafish embryos early in development and behavioral analysis was performed to observe spontaneous movement, touch evoked tail swimming, and final swimming. If it is not yet clear if GABA is required for spontaneous movement or touch evoked tail cell locomotion but our results indicate that GABA strongly modulates swimming behavior. In a second approach to examine the role that GABA plays during embryogenesis, we are analyzing the spatial and temporal expression of GABA receptors. Using DHF databases, we have identified three GABA A receptor γ subunits, each with a high degree of sequence similarity to mammal. One of these subunits, GABA A γ2, has been cloned and sequenced. RT-PCR was performed using mRNA at different stages. These analyses indicate that this GABA receptor subunit is expressed throughout early zebrafish development. We generated an RNA probe and performed in situ hybridizations at 48hpf. Our results were inconsistent, and we are now making a more specific probe. In the future, we will also clone the other γ subunits and perform in situ. Taken together, these experiments will provide a deeper understanding of the role of GABA in the development of the vertebrate nervous system.

Introduction

In early stages of mammalian development, GABA, an inhibitory neurotransmitter, is essential for normal development. In early stages of mammalian development, GABA, an inhibitory neurotransmitter, is essential for normal development. In the second approach to examine the role that GABA plays during embryogenesis, we are analyzing the spatial and temporal expression of GABA receptors. Using DHF databases, we have identified three GABA A receptor γ subunits, each with a high degree of sequence similarity to mammal. One of these subunits, GABA A γ2, has been cloned and sequenced. RT-PCR was performed using mRNA at different stages. These analyses indicate that this GABA receptor subunit is expressed throughout early zebrafish development. We generated an RNA probe and performed in situ hybridizations at 48hpf. Our results were inconsistent, and we are now making a more specific probe. In the future, we will also clone the other γ subunits and perform in situ. Taken together, these experiments will provide a deeper understanding of the role of GABA in the development of the vertebrate nervous system.

Methods

Pharmacological/Behavioral Analysis

GABA receptors at the cell-surface are pressure injected with injection buffer, gabazine (10mM) or muscimol (10mM) 20 minutes prior to observation. The same stage of development was used for all experiments. Two independent experiments were used to control for the variability in the results. In one experiment, the number of control embryos was counted and analyzed. The number of control embryos was counted and analyzed. In another experiment, the number of control embryos was counted and analyzed. The number of control embryos was counted and analyzed.

Molecular Analysis

RT-PCR was performed using mRNA at different stages. These analyses indicate that this GABA receptor subunit is expressed throughout early zebrafish development. We generated an RNA probe and performed in situ hybridizations at 48hpf. Our results were inconsistent, and we are now making a more specific probe. In the future, we will also clone the other γ subunits and perform in situ. Taken together, these experiments will provide a deeper understanding of the role of GABA in the development of the vertebrate nervous system.

Results

1. At 10hpf, gabazine does not affect spontaneous movement, but muscimol inhibits tail-coiling behavior. Pretreatment of zebrafish embryos with muscimol at 10hpf was found to be effective in reducing the number of tail-coiling events.

2. Neurotransmitter-related behavior is also observed throughout development. Throughout development, we observed a decrease in the number of tail-coiling events.

Conclusions

•Both gabazine and muscimol disrupt swimming behavior
•Muscimol also inhibits spontaneous movement and touch-evoked tail cell locomotion

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References


