Localization of Nitric Oxide Producing Cells in the Inferior Colliculous of Mouse Brain

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UNIVERSITY HONORS PROGRAM

SENIOR PROJECT - APPROVAL

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PROJECT TITLE: Localization of Nitric Oxide Producing Cells in the Inferior Colliculus of Mouse Brain

I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

Signed: [Signature], Faculty Mentor

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Comments (Optional):
Localization of Nitric Oxide Producing Cells in the Inferior Colliculus of the Mouse Brain

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4/30/04
Abstract

Nitric Oxide (NO) is a gas that functions as a neurotransmitter in the central nervous system. Nitric Oxide Synthase (NOS) is the enzyme that synthesizes NO from arginine. Once produced, NO diffuses through the cell membrane and surrounding tissues where it functions to modulate the release of other neurotransmitters (e.g., gamma-aminobutyric acid and acetylcholine) via the activation of guanylyl cyclase. NO-producing cells have been found responsible for analyzing olfactory and visual information. Electrophysical studies have shown that NO modifies the stimulus-induced output of individual neurons comprising these centers and hence, plays a role in the neural analysis of olfactory and visual information. While these studies have shown a general role for NO, its function in other sensory systems is not known. Because of this, a series of studies focused on identifying the role of NO in the auditory system have been initiated. One of these, the results of which are presented here, involves the identification of putative NO-producing cells in the inferior colliculus (IC), an important auditory center involved in sound recognition and localization. To accomplish this, paraformaldehyde-fixed sections (50 micrometers thickness) of the mouse (Mus musculus) IC were subjected to NADPH-diaphorase histochemistry to reveal the presence of neurons containing NOS. A large number of labeled neurons were observed in two subdivisions of the IC, specifically, the dorsal cortex and commissural nucleus. Fewer neurons were observed in the external cortex of the IC, while the central nucleus showed little, if any, labeling. Results provide an anatomical substrate supporting a proposed role for NO in auditory function.
**Introduction**

Nitric Oxide (NO) is a gas that functions as a neurotransmitter in the central nervous system. Nitric Oxide Synthase (NOS) is the enzyme that synthesizes NO from arginine. Once produced, NO diffuses through the cell membrane and surrounding tissues where it functions to modulate the release of other neurotransmitters (e.g., gamma-amino butyric acid and acetylcholine) via the activation of guanylyl cyclase. NO-producing cells have been found responsible for analyzing olfactory and visual information. Electrophysical studies have shown that NO modifies the stimulus-induced output of individual neurons comprising these centers and hence, plays a role in the neural analysis of olfactory and visual information. While these studies have shown a general role for NO, its function in other sensory systems is not known. Because of this, a series of studies focused on identifying the role of NO in the auditory system have been initiated. One of these, the results of which are presented here, involves the identification of putative NO-producing cells in the inferior colliculus (IC), an important auditory center involved in sound recognition and localization.

The NADPH-diaphorase (NASPH-d) histochemical reaction is based to the reaction by an enzyme of a tetrazolium salt to an insoluble formazan. This reaction happens when NADPH is present. This reaction has been used to stain the Inferior Colliculus (IC) of the mouse brain.

It has been found that NADPH-d is a Nitric Oxide Synthase (NOS) (Bredt). One can therefore say that NADPH-d positivity represents a cell that produces Nitric Oxide (NO). The mouse was chosen because this part of the brain has been found to be very similar to humans (Vincent).
Methods and Materials

Four mice (weight 30-38g) were used in this study. The animals were
anaesthetized with urethane (1.5g/kg i.p.). They were then perfused transcardially with
50ml of 0.1M PBS (buffer) then 100ml cold 4% paraformaldehyde and 0.1%
glutaraldehyde in 0.1M PBS. The second solution is used as a fixative. Brains were
removed from the skull, postfixed for 90 minutes, and stored overnight in 30% sucrose
in 0.1M PBS at 4 deg. C. Brains were embedded in glutaraldehyde-fixed egg yolk, frozen
on dry ice, then sectioned at 60 micrometers.

The sections were then stained using NADPH-d histochemistry. The sections
were incubated in a solution for around 30 minutes containing 1mg/ml mitroblue
tetrazolium, 0.5mg/ml NADPH, 1.25 mg/ml monosodium malate and 0.6% Triton X-100
in 0.1M PBS. This reaction takes place in the dark at 37 degrees C. Rinsing sections in
0.1M PBS stops the reaction. The slides were then mounted, dehydrated, and
coverslipped.

Results

The NADPH-d cells that stained positive are stained dark blue at the cell body
with axons and dendrites stained the same color going off the cell body. The thickness of
the slides makes it difficult to determine which are axons and dendrites and where they
are moving.

The dorsal cortex is where the majority of staining was found. The dorsal cortex
has been shown to be a major site of the IC where the auditory cortex projects (Drug.). It
has also been shown that NO producing cells have been located in the dorsal cortex of the
IC in rats (Drug.). All of the sections collected showed extensive staining in the dorsal
The commissural nucleus was the site of the IC with the most staining after the dorsal cortex. The commissural nucleus has been shown to be the site of communication between the two parts of the IC (Frisina and Walton). The commissural cortex was not noted as being found to have NO producing cells in similar projects.
Discussion

NADPH-d positivity was found mostly in the dorsal cortex and the commissural nucleus. There were random cells seen throughout the external cortex but with no regularity or order. These results were in line with Druga and with another study conducted by Aschoff. Our results differed in the fact that we found many NO producing cells in the commissural nucleus.

Neurons in the dorsal cortex project to the dorsal cortex and central nucleus on the other side of the brain. The dorsal cortex receives descending information from the auditory cortex. This input is glutamatergic in nature, and activation of the glutamate NMDA receptor permits the entry of calcium into the cell, activating NOS and hence, NO production. The fact that a large number of dorsal cortex neurons show NADPH-diaphorase labeling and hence, probably produce NO - suggests that NO may play a role in the descending cortical modulation of IC output.

Nitric Oxide has been of much interest to everybody in biochemical research because of its unique properties. It has a retrograde action that has been shown to
function to increase the release of other neurotransmitters. This is how NO is very useful in learning and memory. The fact that NO is also a gas has interested many scientists.

Figure 4. This picture shows the auditory pathway. The importance of the IC can be seen here.

Conclusion

The purpose of the series of experiments started here is to identify the role of NO in the auditory pathway. The purpose of this experiment was to identify NO-producing cells in the IC of the mouse brain. The NO-producing cells were found to be present in the dorsal cortex and the commissural nucleus. The external cortex did have some NO-producing cells in it but not to the extent of the other sites.
Bibliography


