Megjegyzés [RT1]:

Detection and neurochemical regulation of the neuronal nitric oxide synthesis in molluscan neuronal networks

PhD Theses

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DETECTION AND NEUROCHEMICAL REGULATION OF THE NEURONAL NITRIC OXIDE SYNTHESIS IN MOLLUSCAN NEURONAL NETWORKS

INTRODUCTION

Nitric oxide (NO) is an evolutionarily conserved signal molecule which mediates several function of the nervous system. NO is produced in a five electron oxidation of the amino acid L-arginine to L-citrulline by nitric oxide synthase (NOS). NOS containing cells are involved in the central ganglia of nemathelminths, annelids, gastropods, cephalopods, crustaceans, insects, chelicerates and echinoderms (Eloffson et al. 1993, Dawson & Dawson 1995, Martínez 1995, Jacklet 1997). The highly diffusible NO can enter an adjacent target cell as an intercellular messenger molecule and enhance the production of the second messenger cyclic guanosine monophosphate (cGMP) from guanosine triphosphate. Neuromodulation and neuroprotection via the NO/cGMP signaling mechanism is essential for some processes of the chemoreception and motor control, furthermore the synaptic potentiation in learning of the invertebrate nervous systems. In vertebrates, tissue specific NOS isoforms, such as endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) are known. Some invertebrate-type NOS isoforms have been also isolated and characterized (Huang et al. 1997).

The aim of this study

The balance of enzymatic NO production is a crucial step in the NO signaling because NO can not be stored in neurons since its high ability to diffusion through biological membranes. Neuronal NOS metabolises L-arginine to NO and L-citrulline in a Ca^{2+}/calmodulin dependent manner in vertebrates, therefore the elevation of intracellular Ca^{2+} concentration through glutamate activated NMDA type Ca^{2+} channels or via nicotinic acetylcholine receptor activation is a key trigger of NO synthesis. In the insect brain not only the activity but also the intracellular localization of NOS is influenced by cytosolic Ca^{2+} concentration. In bivalvian hemocytes, morphine induced Ca^{2+}-transients can contribute to enhaneced NO liberation (Mayer & Andrew 1998, Nieto-Fernandez et al. 1999). Although known gastropod type NOS enzymes share some structural homologies with the mammalian type nNOS they possibly liberate NO in a Ca^{2+} independent way.

Although we know the distribution of NOS and partially the function of NO in the nervous system, we have little information on the neurochemical signals affecting the NOS catalytic activity.

The aim of my study is to describe extracellular signals leading to enhanced or decreased NOS activity by histochemical and biochemical methods.

MATERIALS AND METHODS

In this work we used the central ganglia of *Helix lucorum* (Pulmonata, Gastropda) and the enteric neuronal networks of several pulmonate snails, as alternative experimental paradigms.

The NOS-containing neurons were visualized by the standard NADPH-diaphorase (NADPH-d) histochemical reaction (Nakos & Gossrau 1994). The NO production of isolated tissues were detected by the colorimetric measurement of the NO derived nitrite (Marzinzig et al. 1997, Guevara et al. 1998, Borcherding et al. 2000).

An immunohistochemical method was used to label the invertebrate type neuropeptide FMRFamide-containing nerve cells. The effects of L-arginine, conventional NOS inhibitors, FMRFamide, furthermore FMRFamide receptor blocker amiloride hydrochloride were tesetd on the NO production of neural tissues. The effects of L-arginine and NOS inhibitors were recorded on the isometric tension changes of intestinal muscles by an isometric transducer system.

CONCLUSIONS

The mian findings of the present dissertation are summarized below:

(1) The topography of NADPH-d labeled, NOS-containing neurons and enteric networks was described in the central ganglia of *Helix lucorum* and in the enteric nervous system of several families of the Pulmonate subclass.

(2) The L-arginine dependent nitrite production was detectable both in the central ganglia and in the enteric networks. The nitrite formation was confined to the NADPH-d positive neural tissues and was reduced by conventional NOS inhibitors. Therefore the nitrite production of NADPH-d reactive tissues can be considered as an indicator of their NOS activity.

(3) Anatomical connection was found between NOS containing and FMRFamide producing central neurons in *Helix lucorum.* The FMRFamide containing varicosities around the nitrergic cell surfaces can be the sites of an FMRFamidergic transmission to NO liberating cells.

(4) The intraganglionic NOS activity could be enhanced by FMRFamide. The known FMRFamide-receptor blocker amiloride-hydrochloride abolished the effect of FMRFamide.

(5) The midintestinal motor activity could be influenced by L-arginine and NOS-inhibitors. The NO seems to be a smooth muscle relaxant agent in the investigated organisms.

(6) In the hipometabolic dormant states of *Helix lucorum*, the enteric NO synthesis was blocked, although the organization of the nitrergic network was not altered during the resting periods. In dormancy, L-arginine could not influence the enteric motor activity.

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