Dynamic In vivo Imaging of NADPH Oxidase Gene Expression to Monitor its Involvement in Morphine’s Actions

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ABSTRACT

The present study examined the applicability of using in vivo molecular imaging to monitor the daily expression of locally transfected NADPH oxidase 4 (Nox4) gene in periaqueductal gray (PAG) of mice. A mixture of luciferase expression plasmid and transfection reagent with vectors containing Nox4 was injected into the lateral ventricle at 2 mm rostral and 2 mm lateral with a 45° angle from the bregma. Luciferase expression was monitored daily using an IVIS 200 in vivo imaging system. The in vivo bioluminescent signals were detectable as early as 24 hours after gene transfection into mouse brain and were maintained for 7 days. On days 2 to 7, the antiinvasive efficacy of morphine was significantly increased in Nox4 vector-injected compared to empty vector-injected and naive mice. After in vivo monitoring the mice were euthanized, and brain slices were obtained for ex vivo imaging. Strong signals of transduction of luciferase genes were seen in the PAG area. Real-time RT-PCR, Nox4 mRNA abundance was also found doubled in the dissected PAG, but not in the brain cortex. These results show that the in vivo imaging system used in these experiments provides a powerful tool for daily non-invasive dynamic visualization of gene expression and will be useful for future studies of the role of other genes in drug effects in the brain. (Supported by DK45927, DA10417, DA028051 and HL89165).

METHODS

Animals. Male Swiss Webster mice (Harlan Laboratories, Indianapolis, IN) weighing 25–30 g were housed 6 to 10 cages in animal care quarters and maintained at 22 ± 2 °C on a 12-h light-dark cycle. Food and water were available ad libitum. The mice were brought to a test room (22 ± 2 °C, 12-h light-dark cycle), marked for identification and allowed 10h to recover from transport and handling. Protocols and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Virginia Commonwealth University Medical Center and comply with the recommendations of the IASP (International Association for the Study of Pain).

Route of Injection of Gene Constructs into Brain. i.c.v. (intracerebroventricular) injections of gene constructs were performed in mice anaesthetized with Ketamin/Xylazine (65/5 mg/kg bwt, i.p.), and then a horizontal incision was made in the scalp. A free-hand 5 ml injection of mixed vectors (Luciferase + NOX4 + i.v.) (see above:PEF) Transfection Reagent was made 2 mm rostral and 2 mm lateral at a 45° angle from the bregma into the lateral ventricle. The vectors were diffused and transfected into PAG area via lateral ventricle.

In vivo imaging. Mice were anaesthetized with Ketamin/Xylazine (65/5 mg/kg bwt, i.p.), and a solution of luciferin (150 mg/kg intraperitoneally) (Xenogen Corp.) was injected 5 minutes prior to imaging. The live animals were imaged using the IVIS200 in vivo imaging system (Xenogen Corp.). A grayscale body surface reference image (digital photograph) was taken under weak illumination. After switching off the light source, photons emitted from luciferase-expressing cells within the animal body and transmitted through the tissue were quantified over a defined period of time ranging up to 5 minutes using the software program “Living Image” (Xenogen Corp.) as an overlay on Image (Xenogen Corp.). Raw values are reported as photons/s/cm²/steradian.

RNA extraction and real time RT-PCR. Total RNA was isolated from brain cortex and PAG using TRIzol reagent (GIBCO, Life Technologies, Carlsbad, CA) according to the protocol described by the manufacturer. The mRNA levels for target genes were analyzed by real-time quantitative RT-PCR using a Bio-Rad Cycler system (Bio-Rad, Hercules, CA). The mRNA level was normalized to the 18S mRNA.

Tail immersion test. A simple, inexpensive optical system using blue luminescent or fluorescent protein as a reporter is currently under development. It can visualize gene expression in small living animals in a sufficiently rapid way that it can be used as a daily video recording for real-time measurements in laboratories.

RESULTS

Monitoring gene expression in whole animals is of importance in studies on gene functions. However, before in vivo molecular imaging techniques were developed, determination of gene expression relied on detection of mRNA and corresponding protein levels in fixatives or cells. Since these measurements were usually done by sacrificing animals after functional studies, observed functional changes may not be associated with the gene expression levels throughout the experimental period.

Recently, several in vivo molecular imaging systems were developed to dynamically monitor transgene expression in living animals, such as CT (X-Ray Computed Tomography) and MRI (Magnetic Resonance Imaging). Although these methods are useful under certain conditions for detection of in vivo gene expression, their high cost, radioactive hazard and complicated operation limit the usage in laboratories.

Background

A simple, inexpensive optical system using blue luminescent or fluorescent protein as a reporter is currently under development. It can visualize gene expression in small living animals in a sufficiently rapid way that it can be used as a daily video recording for real-time measurements in laboratories.

SUMMARY

In vivo imaging can be used to monitor local gene expression in brains such as PAG, which could be observed daily in living mice.

Co-transfection of NADPH oxidase isoform, NOX4 by i.c.v. transfection into PAG will provide an approach to investigate the role of NOX4 in morphine responses.

CONCLUSION

The IVIS 200 in vivo imaging system provides a powerful tool for a daily non-invasive and dynamic visualization of gene expression in mouse brain, which can be treated in specific brain regions using i.e.c. injection. This dynamic monitoring of gene expression may guide functional studies of gene function such as NOX4 in morphine responses.