

INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 3 (IGFBP-3) IN THE BRAIN OF MOTHER RATS

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INTRODUCTION

The postpartum physiological and behavioral changes are important parts of reproduction regulated by a complex neuronal network, which includes parts of the hypothalamus. Lesions of the preoptic area abolish maternal behaviors while dopaminergic neurons in the arcuate nucleus regulated by suckling-responsive peptidergic neurons in the posterior intralaminar complex of the thalamus (PIL) regulate prolactin release required for lactation (Cservénák et al, 2010, 2013).

In our previous microarray study, gene expression alterations were described in the anterior hypothalamic areas of mother rat. The highest elevation was shown by the amylin, which we identified as a novel neuropeptide expressed exclusively in the maternal preoptic area (Szabó et al., 2012). The expression of insulin-like growth factor binding protein-3 (IGFBP-3) also increased significantly in lactating mothers as compared to mothers whose litter was taken away immediately after birth. In the blood, IGFBP-3 is the major carrier molecule of insulin-like growth factors (IGFs). It forms a ternary complex with an acid-labile subunit of either IGF-1 or 2. The presence and function of the IGF-system including IGFBP-3 have not been established in the brain. As a first step, we investigated the expression and distribution of IGFBP-3 in the control female and maternal brain.

METHODS

Dissection of the preoptic area

A brain region including the preoptic area was dissected at the 11th postpartum day from the brains of mothers rearing their pups (at least 8 pups) and from the brain of mothers who were separated from their pups immediately after delivery so that their maternal behavior disappears by the 11th day. The preoptic area was dissected bilaterally with parasagittal and horizontal cuts. In the RT-PCR study, 8 mothers with pups and 8 pup-deprived mothers were used.

Real-time RT-PCR analysis

Dissected tissue was kept at -80°C before total RNA isolation with Trizol reagent. RNA was treated with DNase I, and cDNA was synthesized with the Superscript II reverse transcriptase kit (Invitrogen) while PCR was performed with Immomix (Bioline) using SYBR dye. The PCR products were regularly run on gels to check for potential genomic DNA contamination.

In situ hybridization histochemistry

Serial coronal sections (12 - 20 µm thick) were cut and mounted on positively charged slides. [³²S]UTP-labeled riboprobes were used for hybridization at 1 million DPM activity per slide. Subsequently, the slides were dipped in NTB nuclear track emulsion and stored at 4°C for 3 weeks. Then, the slides were developed and coverslipped for microscopic analysis.

Immunohistochemistry

Immunostaining was performed following hybridization using the ABC-peroxidase method visualized with DAB reaction using anti-tyrosine hydroxylase and anti-TIP39 antibodies. The sections were dipped in emulsion after the completion of the immunohistochemistry procedure.

Microscopy and photography

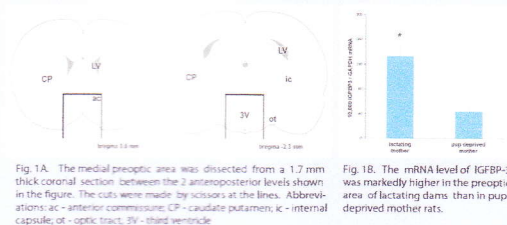
Sections were examined using an Olympus BX60 light microscope. Images were captured with a SPOT Xplorer digital CCD camera.

ACKNOWLEDGEMENTS

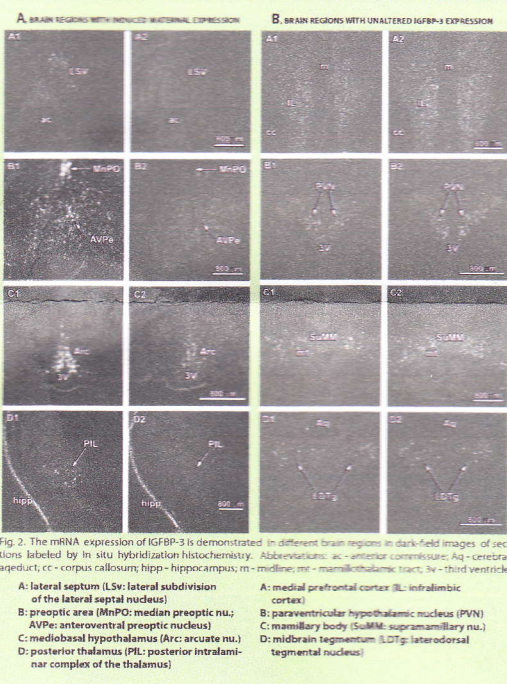
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1. MATERNAL INDUCTION OF IGFBP-3 IN THE DISSECTED PREOPTIC AREA - RT-PCR DATA



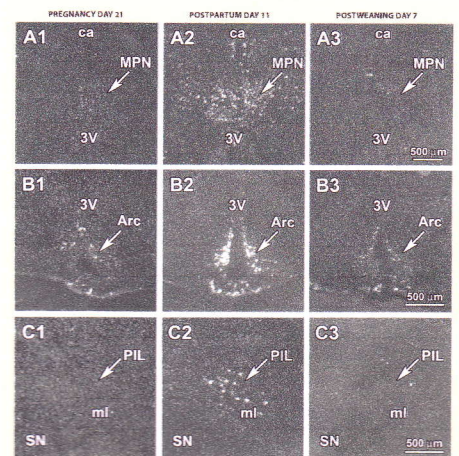
2. THE DISTRIBUTION OF IGFBP-3 EXPRESSION IN THE BRAIN



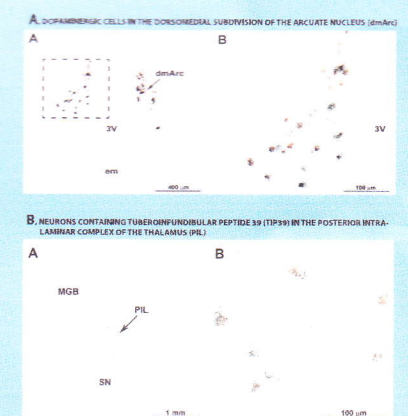
RESULTS

Our previous microarray finding on the increase in the preoptic expression level of IGFBP-3 in mothers was validated by RT-PCR. In situ hybridization histochemistry also confirmed the maternal induction of IGFBP-3 in the preoptic area and revealed increased expression in the median, medial, and anteroventral preoptic nuclei. Additional brain regions, such as the ventral subdivision of the lateral septal nucleus, the arcuate nucleus, and the posterior intralaminar complex of the thalamus (PIL) also demonstrated maternally elevated levels of IGFBP-3 mRNA. The time course of alterations in the IGFBP-3 mRNA levels during the reproductive cycle was also determined: IGFBP-3 mRNA level is low in control female rats, does not elevate by the end of pregnancy but is markedly induced postpartum, and finally, its expression is reduced to the basal level 7 days after weaning of the pups. IGFBP-3 was also abundant in some additional brain regions, including the infralimbic cortex, the hippocampus, the paraventricular hypothalamic nucleus, the supramammillary nucleus, the laterodorsal tegmental nucleus, the area postrema, and the choroid plexus. In these regions, however, the expression of IGFBP-3 was not altered in mothers. We also discovered co-localization of IGFBP-3 with tyrosine-hydroxylase (TH) positive neurons in the arcuate nucleus and with tuberoinfundibular peptide 39 (TIP39) in the PIL using a combination of in situ hybridization and immunohistochemistry.

3. TEMPORAL PATTERN OF IGFBP-3 EXPRESSION DURING THE REPRODUCTIVE CYCLE



4. IDENTIFICATION OF SOME SPECIFIC TYPES OF NEURONS THAT EXPRESS IGFBP-3 IN MOTHERS



CONCLUSION

These data first demonstrate the expression pattern of IGFBP-3 in the brain. Moreover, its induction in some specific brain regions of mother rats suggests some particular role of IGFBP-3 in adaptations of the female brain to motherhood.

LITERATURE

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