学 位 論 文 の 要 旨

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学 位 論 文 名　SPATIAL AND TEMPORAL PATTERNS OF EXPRESSION OF MELANOCORTIN TYPE 2 AND 5 RECEPTORS IN THE FETAL MOUSE TISSUES AND ORGANS

発 表 雑 誌 名
（巻, 初頁～終頁, 年）　Anatomy and Embryology (in press)

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論 文 内 容 の 要 旨

INTRODUCTION

Melanocortin type 2 receptor (MC2R) is a receptor specific to adrenocorticotropic hormone (ACTH). MC2R is expressed most abundantly in the adult mouse adrenal cortex in both the zona fasciculata and glomerulosa, allowing the release of both glucocorticoid and mineralocorticoid by the ACTH effect. Melanocortin type 5 receptor (MC5R) is mainly specific to ACTH and α-melanocyte stimulating hormone (α-MSH). In adult mice, MC5R is expressed at high levels in such exocrine tissues as the lacrimal, Harderian, and sebaceous glands, and is also detected in the adrenal gland and the brain.

Recently, it was suggested that ACTH is related to the prenatal development of mammals. Levels of circulating ACTH were relatively high in fetal mice but declined significantly after birth. We previously showed that AtT20 cells, an ACTH-secreting tumor cell line, transplanted into mouse embryos elevated the plasma ACTH level and changed the morphology and steroidogenesis of the adrenal cortex.

In the present study, we analyzed the expression of MC2R and MC5R in mouse embryos from the late organogenetic period to term by immunohistochemistry, and show that both receptors are differentially expressed in a much wider variety of embryonic tissues and organs than previously thought. Possible functional significances of the expression are also discussed in some of these tissues and organs.

MATERIALS AND METHODS

Animals and tissue preparations

Jcl:ICR mice were used for the present study between 10 and 20 weeks of age. They were mated from 5 pm to 8 am. Noon on the day the vaginal plug was found was calculated as embryonic day (E) 0.5. Whole embryos (E11.5 - E16.5) and organs from E18.5 embryos were fixed in 4% formaldehyde/70% methanol at 4°C overnight, dehydrated, and embedded in paraffin. Sagittal sections (5 μm) of the whole embryos (E11.5 - E16.5) and sections of the organs (E18.5) were placed on slides coated with poly-L-lysine. Some adult organs were prepared similarly.

Antibodies and immunohistochemical detection

Immunohistochemical staining for MC2R and MC5R was performed as follows. Sections were deparaffinized and treated with 3% hydrogen peroxide at room temperature for 5 min. Sections were then microwaved in 10 mM citric acid solution (pH 3.0) for 5 min to boil, blocked with 10% normal goat serum at room temperature for 50 min, incubated overnight at 4°C with primary antibodies, and developed using a standard avidin-biotin-peroxidase complex method. Primary antibodies were rabbit anti-mouse MC2R and MC5R.

Co-localization of MC2R and the mouse germ cell-specific Y-box protein 2 (MSY2), a specific marker for oocytes, in the fetal ovary, and that of MC5R and megalin, a specific marker for renal proximal tubules, in the metanephros, were detected by indirect immunofluorescence using a confocal laser scanning microscope. We used a goat anti-rat megalin polyclonal antibody and a goat anti-MSY2 polyclonal antibody. The secondary antibody for MC2R and MC5R was a goat anti-rabbit IgG-biotin followed by a streptavidin-Cy3, and that for megalin and MSY2 was an anti-goat IgG-FITC.

RESULTS AND DISCUSSION

Both receptors were expressed in the fetal mouse adrenal gland and testis from E13.5 to E18.5. In the genital ridge, MC2R, but not MC5R, was expressed at E11.5 and E12.5. MC2R was expressed in the ovary for several days in different distribution patterns, while MC5R was not detected. In the mesonephros, both receptors were detected at E11.5 and E12.5. In the metanephros, both receptors were observed on different embryonic days (MC2R from E12.5 to E18.5, MC5R from E14.5 to E18.5). In the lung, MC2R was detected from E11.5 to E14.5, while MC5R was not. In blood cells, liver, and nasal epithelium, MC5R, but not MC2R, was detected, especially strongly in blood cells. In the telencephalon, diencephalon, mesencephalon, metencephalon, myelencephalon, and spinal cord, MC2R was detected on the

earlier days (E11.5 to E13.5), while MC5R was not. In the telencephalon, MC5R was detected only on the later days (E16.5, E18.5). In the choroid plexus, MC2R was strongly expressed at E13.5 and E14.5 and weakly from E15.5 onward, while MC5R was not observed at all. Both receptors were present in the dorsal root ganglion from E11.5 to E15.5 (MC2R) or E16.5 (MC5R) and in the trigeminal ganglion from E13.5 to E15.5 (MC2R) or E16.5 (MC5R).

In the testis, MC2R and MC5R were expressed in the spermatogonia and mesenchymal cells from E13.5 to E18.5, but not those in the adult. These findings suggest that ACTH may be related to division of spermatogonia during the fetal period *via* MC2R and/or MC5R.

In the ovary, MC2R was expressed from E13.5 to E18.5, whereas MC5R was not detected at all. We performed double-staining analysis using antibodies against MSY2, as an oocyte marker, and MC2R. Since both proteins were co-localized, MC2R was expressed in the oocytes at E18.5, suggesting that ACTH can affect fetal mouse oocytes *via* MC2R during the oogenesis including meiosis.

In the metanephros, MC2R was expressed in the metanephric tubules from E12.5 to E18.5, and was also expressed in the mesenchyme from E12.5 to E13.5. MC2R expression was localized in the epithelium of the collecting tubule and the mesenchymal cells of the interstitial tissue from E12.5 to E13.5. However, MC2R was not expressed in the mesenchyme after E14.5 and was restricted to the basal and lateral sides of the epithelial cells in the proximal and distal tubules, especially in the distal tubule. On the other hand, MC5R was strongly expressed on the brush border of the epithelial cells in the proximal tubules from E14.5 onward. In the double staining of E16.5 metanephros for the megalin and MC5R, both proteins were co-localized at the brush border of the proximal tubular epithelium. The present findings suggest that ACTH affects the function of the fetal metanephros, *i.e.*, reabsorption and secretion in the proximal tubules and/or distal tubules *via* MC2R and MC5R.

The expression of both receptors showed differential stage- and tissue-specific patterns. In particular, MC2R showed a widespread tissue distribution during developmental stages, unlike in the adult period. These results suggest that ACTH may affect a variety of tissues and organs in mouse embryos.

CONCLUSION

The present study shows the spatio-temporally specific expression patterns of MC2R and MC5R in the mouse embryo and suggests that ACTH may be related to histogenesis and/or prenatal functions of various tissues and organs *via* MC2R and/or MC5R.