immunoreactivity for insulin, while α cells increased in number. The second part of the study aimed to elucidate the difference, with respect to the existence of a CaBP, which is known to buffer Ca^{2+}rises following stimulation. Calbindin-D 28 k is a predominant calcium-binding protein contained in the pancreatic islets. It can buffer Ca^{2+}rises following stimulation and thereby protect cells against calcium toxicity.

The immunoreactivity for calbindin-D 28 k was localized only in α cells in normal mice, but not present in β cells. This finding held true in islet cells of the KATP channel-deficient mice. An immunohistochemical survey using six rodents including the mouse showed that calbindin-D 28 k was preferentially localized in α cells in the rat and guinea pig, while in the hamster both α and β cells were rich in calbindin-D 28 k. None of the α and β cells in the squirrel or gerbil pancreas were immunoreactive for calbindin-D 28 k. This finding may explain how α cells but not β cells in the knockout mice could escape from the calcium toxicity, and shows that the cellular localization of calbindin-D 28 k in the islets differs even among rodents.

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The cellular distribution of estrogen receptor α (ER α) and β (ER β) mRNAs in the reproductive organs of the rat : an *in situ* hybridization study

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This study thoroughly mapped the cellular distribution of estrogen receptor (ER) α and ER β mRNAs in the reproductive organs of the female and male rats by *in situ* hybridization technique in order to elucidate the roles of estrogen in the reproductive events.

In the adult female rat, expression of ER α and ER β mRNAs was predominant in the reproductive tract and ovary, respectively. ER α mRNA had the most pronounced expression in epithelial cells and subepithelial stromal cells from the oviduct to the vagina, while in the ovary it was moderately detected in only the theca folliculi and interstitial glands. The oviduct showed a region-dependent expression pattern of ER α mRNA : the isthmus had the most intense signals while the infundibulum revealed a low intensity of expression. Signals for ER β mRNA in the ovary were most intense in the granulosa cells of healthy follicles whereas degenerating follicles lacked any significant expression. Less intense signals for ER β mRNA were localized in the theca folliculi and corpus luteum. Detectable levels of ER β mRNA were observed in the subepithelial stromal cells from the oviduct to the vagina.

In the developing female reproductive organs, diffuse signals of ER α and β mRNAs were coexpressed in the fetal ovary ; they

were weak and inconsistent before onset of gonadal differentiation, but increased in intensity with age. ER β mRNA signals in the ovary sharply increased in intensity to adult levels by postnatal days 6-7, whereas those of ER α mRNA remained unchanged after birth. ER α was the sole subtype expressed during the prenatal period from the oviduct to the vagina, being localized mainly to the subepithelial stromal cells, and remained predominant thereafter. Signals for ER α mRNA in the epithelia were confined to the oviduct during prenatal and early postnatal periods; those in uterine and vaginal epithelia first appeared by postnatal days 4-5 and 6, respectively. Expressions of ER β mRNA in the reproductive tract were absent during the prenatal period, and were weakly expressed during the postnatal period. Thus, estrogen action in the developing ovary may be comediated by both ER α and ER β, whereas ER α may be the primary mediator in the differentiation and growth of the female reproductive tract.

In the mature male, intense signals for ER α mRNA were expressed exclusively in the epithelia from the efferent duct to the initial segment of the epididymis and in the lamellated corpuscles of the glans penis; moderately in the longitudinal muscles of the vas deferens, accessory glands, epithelium and subepithelial stroma of the urethra. In the developing male, the expression patterns of ER α mRNA in the gonad, efferent duct and initial segment of the epididymis during the perinatal period were essentially similar to those of the adult: ER α signals were expressed most intensely in the epithelia of the efferent ducts and initial segment of the epididymis, and interstitial cells of the testis from prenatal period to adulthood. However, ER α mRNA signals in the primordial epididymis and the vas deferens during prenatal period were confined to the outermost cell-layer of the ducts, whereas thereafter they were only expressed weakly in the epithelium and stroma of the epididymis and moderately in the muscle layer of the vas deferens. In the penis, moderate to intense signals of ER α mRNA were expressed in the mesenchyme of primordial penis, epithelia of penile urethra from prenatal period to adulthood and lamellated corpuscles of the glans penis during late neonatal period. ER β signals were expressed intensely in primordial germ and Sertoli cells during the prenatal period, arterial walls of adult testis, and in the epithelium of the sex accessory glands from the perinatal period to adulthood. The broad expression of ERs in male reproductive organs suggests roles of estrogen in regulating tissue development and the reproductive events.

The presence of ER α and β mRNAs in a broad range of cell-types in the reproductive organs of both sexes, from prenatal period through adulthood, suggests existence of a wide spectrum of estrogenic roles in these cells such as (i) organogenesis, (ii) germ cell, pheromone and steroid production, and (iii) semen concentration and transportation and, (iv) copulation. Furthermore, it implies that ERs may mediate development of tissue abnormalities and infertility resulting from exposure to estrogen-like endocrine disruptors.

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