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Characterization of the guinea pig 3β-hydroxysteroid dehydrogenase/Δ^5–Δ^4-isomerase expressed in the adrenal gland and gonads☆

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Abstract

The guinea pig adrenal gland, analogous to the human, possesses the capacity to synthesize C_{19} steroids. In order to further understand the control of guinea pig adrenal steroidogenesis we undertook the characterization of the guinea pig 3β-hydroxysteroid dehydrogenase/Δ^5–Δ^4-isomerase (3β-HSD) expressed in the adrenal gland. A cDNA clone encoding guinea pig 3β-HSD isolated from a guinea pig adrenal library is predicted to encode a protein of 373 amino acid residues and 41,475 Da. Ribonuclease protection assay suggests that this cDNA corresponds to the predominant, if not the sole, mRNA species detectable in total RNA from the guinea pig adrenal gland, ovary and testis. The guinea pig 3β-HSD shows a similar affinity for both pregnenolone and dehydroepiandrosterone, and in addition, a 17β-HSD type II-like activity was also observed. A phylogenetical analysis of the 3β-HSD gene family demonstrates that the guinea pig is in a parallel branch to the *myomorpha* group supporting the hypothesis that the guinea pig lineage has branched off after the divergence among primates, artiodactyls and rodents, suggesting the paraphyly of the order *rodentia*.

Keywords: Guinea pig; 3β-HSD; Adrenal; Rodent; Phylogeny

1. Introduction

The NAD^+-dependent membrane-bound enzyme 3β-hydroxysteroid dehydrogenase/Δ^5–Δ^4-isomerase (3β-HSD) is the essential enzyme involved in the biosynthesis of all classes of steroid hormones by catalyzing the conversion of the Δ^5-3β-hydroxysteroid precursors pregnenolone (PREG), 17-hydroxypregnenolone (17OH-PREG), dehydroepiandrosterone (DHEA), and androst-5-ene-3β, 17β-diol (Δ^5-diol) into their corresponding Δ^4-3-ketosteroids, namely progesterone (PROG), 17β-hydroxyprogesterone (17OH-PROG), Δ^4-androstenedione (Δ^4-DIONE) and testosterone (T). Its deficiency is responsible for a rare form of congenital adrenal hyperplasia associated with a male pseudohermaphroditism. In mammals, the 3β-HSD isoenzymes are expressed in the classical steroidogenic tissues, namely the adrenal gland and the gonads, the ovary and testis. 3β-HSD is also present in the placenta and several peripheral tissues including the skin, liver, adipose tissue, brain, kidney, epididymis, vas deferens, lung, mammary gland, prostate, bone and cardiovascular tissue, where they are involved in the local intracrine formation of sex steroids (for a recent review, see [1]).

☆ The nucleotide sequence reported in this paper has been submitted to the GenBank™/EMBL data bank with the accession number AY914174.

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Multiple 3β-HSDs have been characterized in rodents, namely the rat, mouse and hamster. The structure of four types of rat 3β-HSD cDNAs which all encode a 373 amino acid protein have been elucidated [2–4]. Six 3β-HSD isoenzymes in the mouse [5–10], and three isoenzymes in the hamster have been cloned [11,12]. In addition, a single 3β-HSD isoenzyme has been cloned in seven other species, namely the macaque ovary [13], the bovine ovary [14], the horse testis [15], the chicken adrenal [16], the rainbow trout ovary [17] and the eel ovary [18] and one type has been identified in databases by blast analysis in the drosophila.

Phylogenetic analyses of the 3β-HSD gene family strongly suggest that the need for different 3β-HSD genes occurred very late in mammals, with subsequent evolution in a similar manner in other lineages. Therefore, to a large extent, the 3β-HSD gene family should have evolved, to facilitate differential patterns of tissue- and cell-specific expression and regulation involving multiple signal transduction pathways, which are activated by several growth factors, steroids and cytokines [1].

The pattern of adrenal steroidogenesis differs largely between mammalian species. While corticosterone is the glucocorticoid produced in rats and mice, due to the lack of adrenal P450c17 expression, cortisol represents the major glucocorticoid in guinea pig, as in humans [19]. Although DHEA and dehydroepiandrosterone sulfate (DHEAS), being the major circulating C_{19} steroids in humans, are not detected in the guinea pig circulation, it has been demonstrated that another C_{19} steroid, namely 11beta-hydroxyandrostenedione (11β-OHDIONE), is produced by the guinea pig adrenals and that the levels of circulating 11β-OHDIONE could be stimulated after administration of adrenocorticotropic hormone (ACTH) [20–23]. These characteristics, therefore, support the notion that the guinea pig is a good model of adrenal steroidogenesis.

The aim of the present study was to clone and characterize the guinea pig 3β-HSD isoenzyme expressed in the adrenal, which will aid in the further understanding of the control of steroidogenesis in the adrenal gland. We have also performed phylogenetic analysis to study the relationship between the guinea pig 3β-HSD and other members of the 3β-HSD family identified to date in order to gain further information about the origin of caviomorphs considering the current controversy regarding this issue.

2. Materials and methods

All the restriction endonucleases, T3 RNA polymerase, oligo(dT) and the sequencing kit were purchased from Pharmacia LKB Technology Inc. The ribonuclease protection assay (RPA) II kit was purchased from Ambion (Austin, TX). The Bluescript KS vector was obtained from Stratagene (La Jolla, CA). The PCR RNA kit and the Taq DNA polymerase were purchased from Applied Biosystems (Foster City, CA). The Hybond N^+ nylon membrane, [α-^{32}P] dCTP (3000 Ci/mmol), and the Megaprime DNA labeling kit were purchased from Amersham (Amersham Pharmacia Biotech Inc. Picastaway, NJ, USA). [^{3}H]-Pregnenolone (21.1 Ci/mmol), [^{3}H]-DHEA (60 Ci/mmol) and [^{3}H]-DHT (50 Ci/mmol) were all purchased from DuPont-New England Nuclear (NEN Life Science Products Inc., Boston, MA). All other reagents were of analytical grade and were purchased from ICN Biochemicals Inc. (Cleveland, OH) or Bio-Rad (Richmond, CA).

2.1. Animals

Adult male guinea pigs (Hartley) weighing 450–500 g and females of 600–650 g were obtained from Charles River Canada, Inc. (St. Constant, Qué., Canada). The animals were kept at a controlled temperature of 22–25 °C with a light period from 05:00 to 19:00 h and given regular diet food and water ad libitum. All animals were sacrificed according to the guidelines approved by the animal care committee. Tissues were quickly removed, trimmed of fat using sterile instruments, immediately frozen in liquid nitrogen, and kept at −80 °C until RNA extraction.

2.2. Construction of the guinea pig adrenal cDNA library and isolation of cDNA clones

Total RNA was extracted from total guinea pig adrenal gland using a modified single-step method based on that of Chomczynski and Sacchi [24]. Poly(A)$^+$ RNA was then isolated by oligo(dT)-cellulose chromatography as previously described [25]. Oligo(dT)-primed cDNA was prepared from 5 μg of the poly(A)$^+$ RNA using an oligo(dT) containing *Not*I sequence. After ligation of *Sal*I adaptors and digestion with *Not*I, the cDNA preparation was fractioned on Sephacryl S-500 HR chromatography columns [26]. Fractions enriched for 1-kb fragments were ligated to lambda-gt-22A-*Sal*I-*Not*I-arms.

The guinea pig adrenal cDNA library constructed in a λgt22 phage vector was then screened with a ^{32}P-labeled rat type I 3β-HSD cDNA probe, by the random primer method previously described by Feinberg and Vogelstein [27]. Prehybridization and hybridization were performed following standard procedures. Among the 27 clones that were isolated, one encodes a full-length guinea pig 3β-HSD cDNA. *Eco*R1 restriction fragments were subcloned into the *Eco*R1/*Kpn*1 restriction site of the Bluescript KS vector and plasmid DNA was prepared by alkaline lysis. Sequencing of the double-stranded plasmid DNA was performed according to the dideoxy chain termination method using a modified T7 DNA polymerase (Sequenase Kit, US Biochemical Corp., OH).

2.3. Transient expression of guinea pig and rat type I 3β-HSD cDNAs

A full-length cDNA insert corresponding to the guinea pig adrenal 3β-HSD was subcloned into the pCMV vector,