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Role of Acyl-CoA Synthetase 4, an Arachidonate-Preferring Enzyme Expressed in Steroidogenic Tissues

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Ⅰ. INTRODUCTION

In mammals, fatty acid utilization is initiated by activation of fatty acid, catalyzed by acyl-CoA synthetase(ACS, EC6.2.1.3). This enzyme reaction is essential in fatty acid metabolism, since mammalian fatty acid synthetase contains a specific thioesterase to produce fatty acid as the final reaction product. Acyl-CoA, the product of ACS, is utilized in various metabolic pathways including membrane biogenesis, energy production and fat deposition.

In previous studies, we identified five rat ACSs, designated as ACS1-5. Although the five enzymes exhibit a structural architecture common to ACSs from various organisms, they can be classified into two subfamilies based on their structures and fatty acid preferences: one consists of ACS1(1), ACS2(2) and ACS5(3), and the other of ACS3(4) and ACS4(5). ACS1, ACS2 and ACS5 are closely related in structure and exhibit similar acyl-chain specificity, but their tissue distributions are completely different. ACS1 mRNA is expressed in liver, heart, and adipose tissue, whereas ACS2 and ACS5 expressed mainly in brain and small intestine, respectively. Between ACS3 and ACS4, approxomately 68% of amino acids are identity but they are show poor amino acid identity with ACS1, ACS2, and ACS5. ACS3 mRNA is expressed highly in brain, and to a much lesser extent in lung, kidney, small intestine, and adipose tissue. The mRNA of ACS4 is expressed predominantly in steroidogenic tissues including the adrenal gland, ovary, and testis. ACS3 activates arachidonate, eicosapentaenoate, laurate, and myristate with highest efficiency, whereas ACS4 prefer a narrow range of fatty acids including arachidonate, and eicosapentaenoate most preferentially.

In arachidonate metabolism, ACS palts a key role in the esterification of free arachidonate into membrane phospholipids, Following its release by the action of calcium dependent phospholipases, free arachidonate is believed to be rapidly converted to arachidonoyl-CoA and reesterified into phospholipids in order to prevent excessive synthesis of eicosanoids. In steroidogenic tissues, arachidonate released by cholesterol ester hydrolase, a key enzyme in steroidogenesis, is believed to play mosulatory roles in steroidogenesis.

Ⅱ. STRUCTURE OF RAT ACS FAMILY

ACS is a member of luciferase family and consists of five common regions: N-terminal region(NH2), luciferase-like region 1(LR1), linker region(linker), Luciferase-like region 2(LR2), and C-terminal region(COOH). ACS1 is well characterized ACS and show highly similar to ACS2 and ACS5. In contrast, ACS3 and ACS4 show poor amino acid identity with ACS1, ACS2, and ACS5. The amino acids in luciferase-like region 1 and 2

show the highest similarities that the two regions are crucial for the catalytic reaction of ACS.

Ⅲ. FATTY ACID SPECIFICITY OF RAT ACS

The ACS1 and ACS2 utilize saturated fatty acids with 10~18 carbon atoms. Among the unsaturated fatty acid with 16~22 carbon atoms, the best substrates were palmitoleate, oleate and linoleate for ACS1, and oleate, arachidonate, eicosapentaenoate and docosahexaenoate for ACS2. The best substrates for ACS5 are palmitate, palmitoleate, oleate, linolate, and linoleate. The ACS3 utilizes a rather wide range of fatty acids including laurate, myristate, arachidonate, and eicosapentaenoate. The relative activity of ACS3 for these fatty acids are ~2-fold higher than those of ACS1, ACS2, and ACS5.

Despite the structural similarity of ACS3 and ACS4, the two enzyme exhibit different fatty acid specificity. ACS4 uses arachidonate and eicosapentaenoate most preferentially among C_8-C_{22} saturated fatty acids and C_{14}-C_{22} unsaturated fatty acids.

Ⅳ. TISSUE DISTRIBUTION OF ACS mRNA IN VARIOUS RAT TISSUES

The ACS1 mRNA is expressed in liver, heart, adipose tissue and, to a much lesser extent, in brain and small intestine. In contrast, ACS2 is mainly expressed in the brain. The ACS3 mRNA is predominantly expressed in the brain and appears, to a much lesser extent, in lung, small intestine, adrenal gland, kidney, epididymis, and ovary but is not detected in heart, liver, and spleen. The ACS5 transcripts are present most abundantly in small intestine and to lesser extent in the lung, liver, adrenal, adipose tissue, and kidney. The ACS4 are expressed in wide rang of tissues, with the highest level in adrenal gland; relatively high levels in epididymis, brain, seminal vesicle, ling, ovary, and liver; and lower levels in adipose tissue, kidney, and testis. A trace amount of the mRNA was detected in skeletal muscle, spleen, uterus, small intestine, and heart. ACS4 may have a key role of arachidonate metabolism in steroidogenic tissues(5).

Ⅴ. IMMUNOHISTOCHEMICAL ANALYSIS OF ACS4

ACS4 expressed predominantly in steroidogenic tissues, expecially adrenal gland and ovary. In adrenal gland, immunoreactivity was detected most intensely in zona fasciculata and reticularis. Immunoreactivity in zona glomerulosa was relatively weak and no significant immunotractivity was observed in the capsule or adrenal medulla. In the ovary, significant immunostaining was detected in the large cells but was not observed in the small cells of corpus luteum. It is also present in stromal luteinized cells of the ovary, but no significant immunoreactivity was observed in nonluteinized stromal cells, surface epithelium, or the great majority of ovarian follicles. In testis, immunoreactivity was observed exclusively in the Leydig cells but not in cells of the seminiferous tubules(5).

Ⅵ. REGULATION OF ACS4 IN THE STEROIDOGENIC CELLS

In steroidogenic cells, cholesterol is actively metabolized to produce steroid hormones under the regulation of ACTH. In rodent, the major source of cholesterol is from cholesterol esters in plasma high density lipoprotein, which contains a high contant of arachidonate in its cholesterol ester fatty acids(6). Despite the high content of arachidonate in cholesterol esters, and the presence of prostaglandin production system, no detectable prostaglandins are

produced in adrenocortical cells in the presence or absence of ACTH. ACTH regulates induction of various genes in steroidogenic cells. In adrenocortical cells, ACTH induced gene expression is mediated predominantly by increases in intracellular cAMP and subsequent activation of the protein kinase A signaling pathway(7).

Previous studies showm that ACS4 protein is expressed at high levels in steroidogenic cells of the rat adrenal, ovary, and testis. To determine if ACS4 expression is regulated in coordination with induced adrenal steroidogenesis, we injected mice with a saline solution with or without ACTH and determined the levels of the ACS4 mRNA and protein in the adrenal, ovary, and liver. Although the levels the mRNA did not changed significantly, approximately 2.5- and 1.5-fold induction of protein was observed in adrenal and ovary, respectively, by ACTH treatment, but no significant change was seen in the liver(8). This observation suggests that ACS4 is indeed regulated in coordination with adrenal and ovarian steroidogenesis and that endogenous ACTH may be responsible for the high basal levels of ACS4 in adrenal and ovarian tissues of untreated animal.

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