14. THE FUNCTION AND DIFFERENTIATION OF OVARIAN MITOCHONDRIA

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In mammalian tissues, mitochondria are the primary sites for the production of adenosine triphosphate (ATP), required for most growth and synthetic activities. For this reason, one would expect mitochondria to play an important role in the physiology of a dynamic organ such as the ovary. Furthermore, not only do ovarian tissues require the energy stored as ATP for growth and differentiation, but they also use ATP for the production of cyclic AMP (cAMP), the second messenger for many gonadotropic actions in the ovary (Marsh 1976). The importance of mitochondria in ovarian physiology is underscored further by the fact that these subcellular organelles are the exclusive sites for the synthesis of pregnenolone from cholesterol, the rate-limiting step of ovarian steroidogenesis. In a sense, therefore, it can be said that the most important functions of the ovary are dependent upon products from mitochondria.

Similar to mitochondria of other steroidogenic tissues, ovarian mitochondria contain two major functional systems, the respiratory chain associated with ATP synthesis, and the cholesterol side-chain cleavage enzyme complex required for pregnenolone synthesis. As is the case for all mitochondria, the respiratory chain of ovarian mitochondria contains flavoproteins and cytochromes b, c, c1, and a,a3, (the major component of cytochrome oxidase (Wainio 1970). In comparison, the cholesterol side-chain cleavage enzyme complex consists of a flavoprotein, a nonheme iron protein, and cytochrome P-450 (Kimura and Ohno 1968). In both of these functional systems, reduction of their components in a sequential fashion is initiated through the oxidation of substrates from the citric acid cycle.

1 RELATIONSHIP BETWEEN ADENOSINE TRIPHOSPHATE SYNTHESIS AND MITOCHONDRIAL STEROIDOGENESIS

It is felt that the normal respiratory chain and the cholesterol side-chain cleavage enzyme complex are linked in some manner through the mitochondrial pyridine nucleotides, NADH and NADPH. NADH is formed by the oxidation of most substrates and then introduces electrons into the respiratory chain, resulting in electron transport. On the other hand, NADPH is of importance because it is the cofactor that introduces electrons to the cholesterol side-chain cleavage enzyme complex. In other mitochondrial systems, H+ can be passed between NAD+ and NADP+ via transhydrogenase enzymes (Wainio 1970).

It has been hypothesized that the normal respiratory chain and the cholesterol side-chain cleavage enzyme complex are interrelated primarily by an energy-dependent transhydrogenase which transfers H+ from NADH to NADP+ (Uzgiris et al. 1971). The energy for this reaction is derived from substrate oxidation and is stored in the form of high-energy intermediates. These hypothesized high-energy intermediates then are used to support the reduction of NADP+ and the synthesis of ATP. In contrast to this hypothesis, there are data which indicate that energy may not be required for mitochondrial steroidogenesis. Rather, it is hypothesized that energy from substrate oxidation may not be essential for the reduction of NADP+ (Robinson and Stevenson 1971). In our studies on luteal mitochondria, we used succinate as a substrate because its oxidation normally does not reduce NAD+ (Dimino et al. 1976). In fact, for succinate to reduce NAD+, energy from its oxidation must be expended to support reversed electron transport.
We found that in-vitro treatment of luteal mitochondria with dinitrophenol (DNP) or carbonyl cyanide fluorophenylhydrazone (FCCP), uncoupling agents that prevent formation of high-energy intermediates (Wainio 1970), abolishes ATP synthesis but only partially inhibits conversion of cholesterol to pregnenolone. These results would support the concept that energy derived from succinate oxidation is required for ATP synthesis but is not essential for cholesterol-conversion activity. However, the problem with these data is that it is difficult to imagine how succinate oxidation could reduce NAD+ and ultimately NADP+ without expenditure of energy.

In another experiment, luteal mitochondria were treated with oligomycin, an inhibitor of the synthesis of ATP but not of high-energy intermediates. The oligomycin treatment of luteal mitochondria causes the cessation of ATP synthesis but also results in a dramatic increase in cholesterol-conversion activity. These findings suggest that energy normally used for ATP synthesis could be diverted to support mitochondrial steroidogenesis. As another approach to this problem, luteal mitochondria were treated with aminoglutethimide, a specific inhibitor of conversion of cholesterol pregnenolone. Aminoglutethimide completely inhibits cholesterol-conversion activity, but causes a modest increase in ATP synthesis.

From these inhibitor studies, we have hypothesized that energy derived from succinate oxidation, while not required, enhances mitochondrial steroidogenesis. At present we cannot explain how succinate oxidation can support pregnenolone synthesis without reversed electron transport. Possibly, an alternate pathway between succinate and the cholesterol side-chain cleavage enzyme complex exists which is energy-independent. At any rate, when energy is made available to support mitochondrial steroidogeneses, it may be utilized to drive reversed electron transport and possibly the energy-dependent transhydrogenase. These concepts are expressed graphically in Figure 1.

II CHANGES IN MITOCHONDRIA: INDICATORS OF FOLLICULAR DIFFERENTIATION

The ovarian follicle progressively develops stero-