High-voltage electron microscopy of adrenal medulla: Direct connections between mitochondria and catecholamine-storage vesicles[1]

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Summary. A high-voltage electron microscopic study of adrenal medullary cells from hypoglycemia-stressed rats revealed the existence of tubular channels which create a luminal continuity between the mitochondrial compartment and the catecholamine-storage vesicles. It is suggested that these channels allow for the transfer of materials such as high-energy nucleotides between the mitochondria and the catecholamine-storage vesicles without an intervening membrane.

Our interest in the association of the mitochondrial compartment with that of other cellular organelles stems from the apparent lack of understanding of the manner in which adenosine triphosphate (ATP) becomes incorporated into storage vesicles of secretory cells. The presence of ATP in storage vesicles is a factor that many endocrine and neuronal tissues have in common[2].

Catecholamine-storage vesicles in adrenal medullary and neuronal tissue contain large amounts of ATP as well as soluble protein and the biologically active amine[3]. Recent studies have demonstrated that ATP synthesis in the adrenal medulla occurs primarily in the mitochondria and the nucleotide is subsequently transferred to the storage vesicles[4]. Results of in vitro studies of the incorporation of ATP into the vesicles are consistent with a specific carrier-mediated process[5] but the evidence is not conclusive. Indeed other studies have demonstrated no or little uptake of ATP into isolated storage vesicles[6,7]. In studies where ATP uptake was measured it was shown to be much less than the rate of uptake measured for catecholamines[5,8]. Since it is well known that ATP is incorporated into the catecholamine-storage vesicles in large quantities, the present study was initiated to explore some of the morphological aspects of this phenomenon.

Materials and methods. Rats were subjected to hypoglycemic shock by the injection of insulin (5 IU/kg b.wt, i.p.). The adrenals were prepared for microscopic evaluation at time intervals (0, 4, 24, 48, 72, 96 h post-insulin) by whole body perfusion followed by immersion in 3% glutaraldehyde. After postfixation in 2% osmium tetroxide the tissue was dehydrated and embedded in a routine manner. Sections 0.5 to 1.0 micron thick were cut on glass knives. The specimens were examined in a high-voltage electron microscope (HVEM) at 800–1000 kV accelerating voltage. Areas selected for study were photographed at different angles. Pairs of micrographs were studied under a lens-mirror stereo viewer which rendered a true 3-dimensional effect.

Results. Study of the micrographs revealed continuities between mitochondrial membrane and that of the catecholamine storage vesicles. In selected micrographs representing 475 μm^2, more than 100 continuities were observed. These continuities, which have not been previously described, appeared to take the form of a tubular extension

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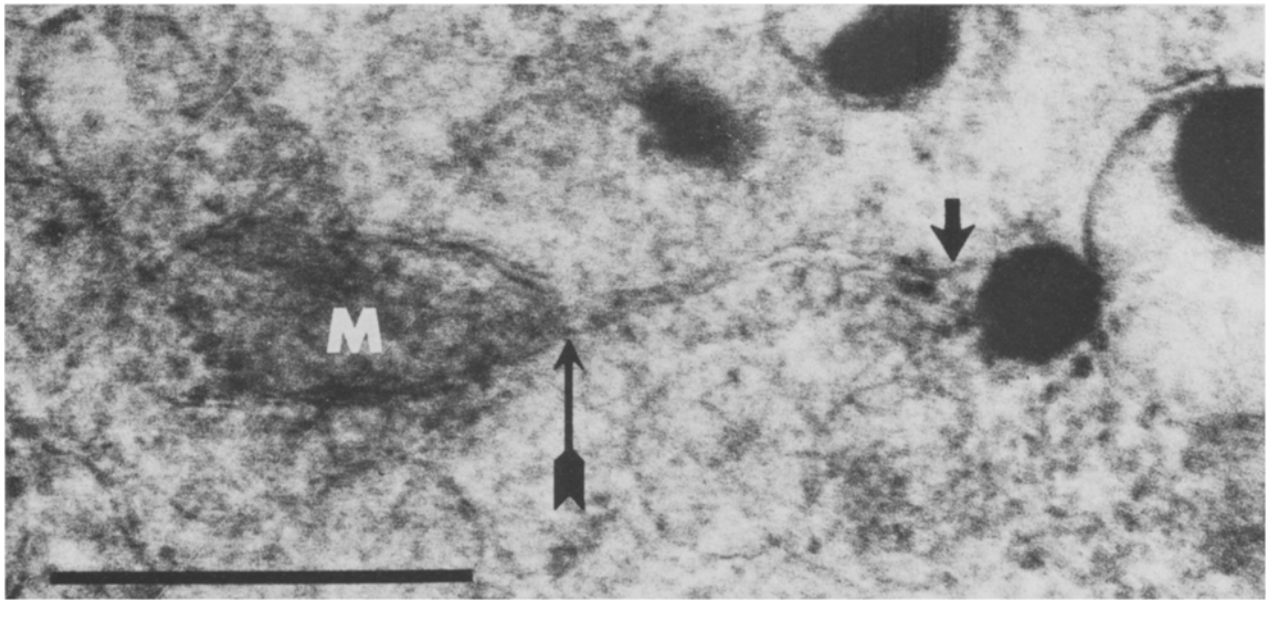

A high-voltage electron micrograph of adrenal medulla from a rat 4 h post-insulin. A tubular connection is seen emerging from a mitochondrion (M) at arrow and extending to surround a catecholamine-storage vesicle (arrowhead). The horizontal bar represents 0.5 μm.

of the outer mitochondrial membrane that extended a variable distance in the cytoplasm to become continuous with the membrane of the catecholamine-storage vesicle (figure). The diameter of the tubular extension was about 20 nm and the length varied from a negligible distance when the vesicle appears to be in direct contact with the mitochondrion, to over 1 μm. Due to the tubular extension, the compartment between the inner and outer mitochondrial membranes was in direct continuity with the vesicle contents without an intervening membrane. These continuities involved about 25% of the mitochondria from tissue of control rats (no insulin treatment) as compared to about 40% of the mitochondria seen in tissue from rats perfused 4 h after insulin treatment and 50% of the mitochondria from rats 24 h post-insulin.

Discussion. It has been established that ATP is synthesized principally in the mitochondria and accumulated in the catecholamine-storage vesicles (see above). Although other mechanisms may be involved, our observations suggest that ATP could be transported from mitochondria to catecholamine-storage vesicles directly through membrane-lined channels. This may occur more often during periods of high synthetic activity such as insulin-induced hypoglycemia and the channels may serve only as a supplemental transport process. The channels were seen to occur more frequently in some cells than others in the same gland. This supports the contention that the reaction of the adrenal medulla to stress tends to exhibit an all-or-none response on the cellular level[9].

Although our observations have been restricted to the adrenal medulla, we may postulate the possibility that direct channels exist in other cellular systems where ATP is transported from mitochondria to other organelles. For example, Droz has suggested that direct contacts between mitochondria of neural tissue and axonal smooth endoplasmic reticulum (SER) may provide direct transfer of materials required for the maintenance of mitochondria during their long transit time from the cell body to the nerve terminal[10,11]. He did not speculate that transfer of high-energy nucleotides from the mitochondria to the lumen of the SER might be occurring, but this would be an interesting possibility since he suggested that the transmitter storage vesicles (presumably rich in ATP[2]) 'pinch off' at terminal arborizations of the SER. This possibility would be of additional interest if ATP were shown to have the properties of neurotransmitter as suggested by Burnstock[12]. As discussed by Volk[13], it would be a fortuitous ultrathin section to allow the visualization of an undulating tubular connection such as the one described in this report, and then the observation would be too uncommon to attach any special significance to it. Indeed, other authors argue that extensions from mitochondrial profiles are artifacts of thin sectioning[14-16]. But the HVEM offers the advantage of greater electron penetration of the specimen, thus thicker sections can be examined with reasonable resolution. Examination of thick sections (0.5–1 μm) on a tilt stage allows a 3-dimensional evaluation of the interrelationship of cell organelles through a greater distance in the tissue. Specifically, the tubular connection that we describe can be visualized in 3-dimensions, ruling out the possibility that the images are the result of superimposition of structures.

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Effect of dimethyl sulfate on the secondary structure of DNA

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Summary. Hydroxyapatite chromatography has been used to demonstrate that alkylation of DNA at neutral pH may lead to denaturation under conditions where no significant depurination occurs. Presence of salt has a preventive effect on such denaturation.

Many chemical carcinogens belong to the class of alkylating agents. Both in vivo and in vitro studies have indicated that principle target of these agents are the nucleic acids[2-4]. Lawley and Brooks have shown that the main alkylation sites in double stranded DNA are the N-7 of guanine and N-3 of adenine[5]. In addition, the alkylation of DNA-phosphates may also occur. However, on this aspect there are conflicting reports in the literature[6,7]. Hsiung et al.[8] have postulated that partial denaturation of alkylated DNA at higher pH values may occur through the disruption of hydrogen-bonding due to a base catalysed imidazole ring opening of the quarternized N-7 guanine. Rizvi and Hadi have earlier published a preliminary experiment showing that alkylation may lead to denaturation of DNA under neutral conditions[9]. To our knowledge, these are the only reports available in the literature on the effect of alkylating agents on the secondary structure of DNA. In the present work we have used hydroxyapatite chromatography to demonstrate the denaturation of DNA as a result of alkylation without causing significant depurination. Dimethyl sulfate has been chosen as the alkylating agent, since it is known to cause minimum alkylation of DNA-phosphates[10].

Materials and methods. Calf thymus deoxyribose-nucleic acid (sodium salt, average mol. wt 1 million) was obtained from Sigma Chemical Company, and was used without further purification. Dimethyl sulfate was obtained from May and Baker Ltd, England. Hydroxyapatite was prepared as described by Bernardi[11].

A 2-mg/ml solution of DNA in TNE (0.01 M tris-HCl, pH 7.4, 0.01 M or 0.1 M NaCl and 2×10^{-4} M EDTA), was methylated by adding sufficient dimethyl sulfate (DMS) to obtain the desired DNA nucleotide/DMS molar ratio. The solution was gently shaken at 25 °C for the desired period of time. The acid released by the hydrolysis of the methylating agent was neutralized by the addition of i.m. NaOH and the pH was maintained between 6 and 7. Depurinated DNA was obtained from alkylated DNA by incubating the latter at 50 °C for 4 h[12]. Hydroxyapatite Chromatography was done as described by Bernardi[11]. To determine the alkali labile acid-soluble