Metabolism and Ultrastructure in Ovaries of Alloxan-Diabetic Juvenile Rats*

E. Stähler, H. O. Neumann, K. M. Niethammer
Universitäts-Frauenklinik Marburg (Lahn)
(Director: Prof. Dr. R. Buchholz)

Received July 15, 1975

Summary. Tests were carried out on the influence of alloxan-induced diabetes mellitus on the metabolism and the ultrastructure of ovaries of juvenile rats. The diabetes mellitus caused the following changes in the metabolism: reduction in the concentration of ATP and NADPH, increase in the lactate/pyruvate quotient to above 40, reduction in the ATP/ADP quotient to below 1, reduction in the level of activity of the hydrogen-conveying enzymes G-6-P-dehydrogenase, isocitrate dehydrogenase and malate dehydrogenase, increase in the level of activity of the alkaline phosphatase, reduction of the protein content.

Ultrastructure: almost complete disappearance of the rough endoplasmic reticulum, shrinkage of the mitochondria, reduction of the cristae and condensation of the matrix. The smooth endoplasmic reticulum remains unchanged, the extent of the Golgi-complex is reduced. Easy removal of the lipid deposits.


Untersuchungen über den Stoffwechsel und die Ultrastruktur in Ovarien alloxaan-diabetischer Ratten


* Die Untersuchungen wurden durchgeführt mit Unterstützung der Deutschen Forschungsgemeinschaft.
Methodology

Juvenile female rats were subcutaneously injected with 12 mg alloxan/100 g body-weight at the age of twenty days. Immediately beforehand alloxan was freshly prepared in a phosphate-citrate-buffer at 4.0 pH in accordance with the guidelines of Klebanoff and Greenbaum in 1964.

The animals were killed after eight days by the effects of ether anesthetic and only those animals were used which showed glucosuria during daily checks. In order to determine the level of blood-sugar, blood was taken from each animal by heart puncture and after removal of the albumen with 0.33 N HClO₄, the blood was analysed according to the hexokinase reaction (optical test Boehringer, Mannheim). The ovaries used for the determination of substrate were immediately immersed into liquid nitrogen and were then homogenised at 4°C with 0.6 NClO₄ in the ultra turrax. Subsequent centrifugeing for ten minutes at 3000 g. ATP, ADP, lactate, and pyruvate were determined by the optical tests of Boehringer, Mannheim, glucose by the hexokinase reaction (Boehringer, Mannheim). NADP and NADPH were determined according to the methodology indicated by Klingenberg in 1970.

The extraction of the ovaries for the determination of the enzymes was carried out at 4°C with a K-Na₂HPO₄ (100 mM) – EDTA (2 mM) buffer at 7.2 pH. After homogenisation additional treatment of the homogenisate with ultrasonic homogeniser (Braun-Melsungen). The determination of the enzymes was carried out with a tolerance of 100,000 g according to the optical tests of Boehringer, Mannheim. The protein content was determined by means of the Biuret method. The ovaries for the electron-microscopic examination were prepared as follows:— preservation at 4°C in 2.5% glutaric aldehyde + 2% formic aldehyde in cacodyl buffer at 7.4 pH (for five hours). Preservation and re-preservation in 1% OSO₄ according to the methods of Karnovsky (1965). The tissue is dehydrated by means of acetone and placed in epone. Silvergrey ultra-thin sections were contrasted with uranyl acetate and lead citrate according to Venable and Coggeshall (1965) and examined with EM 9s of Zeiss, Oberkochen. Luteinized-interstitial gland cells were examined, as these cells are regarded as producers of hormones at that age.

Introduction

Diabetes mellitus is usually connected with an impairment of reproductive functions. The cycle is terminated resulting in permanent dioestrus and a decrease in weight of the ovary (Farina et al., 1971; Liu et al., 1972). The responsiveness of the ovaries to exogenously introduced gonadotrophins which normally causes an increase in weight of the ovaries (Steelman, Pohley, 1953) is clearly impaired (Pivetta et al., 1968). On the third day of gravidity the number of ova being implanted is reduced, and the disturbed fertility also becomes evident through changes of the uterine lipid metabolism (Foglia et al., 1963; Chieri et al., 1969; Foglia et al., 1970; Chieri and Fridhandler, 1965).

Investigations which have been undertaken to throw light on the question of whether the disturbance is primarily in the gonads or is a secondary effect of reduced production and/or release of gonadotrophins have shown that in the case of experimental diabetes mellitus of rats no reduced FSH and/or LH level is found in the plasm of either female (Liu et al., 1972) or male rats (Howland and Zebrowski, 1974). These findings led to the supposition that the diabetic ovaries are no longer in a position to react sufficiently to the stimulus in spite of sufficient gonadotrophin level in the plasm. Studies to clarify the reason for this decreased responsiveness to gonadotrophic hormones have shown that the choles terine content which usually decreases with LH in the ovary (Herbert, 1967) remains largely unchanged in the case of a diabetic ovary (Farina et al., 1971).