Lysosomal enzymes in the juxtaglomerular cell granules

B. M. Soltész, Sz. Gomba and M. Szokol

Department of Pathology, University Medical School Debrecen, H-4012 Debrecen (Hungary), 24 July 1978

Summary. In addition to the already known acid phosphatase and β-glucuronidase, 2 other lysosomal enzymes: aryl sulphatase and N-acetyl-β-glucosaminidase were localized by histochemical methods in the renin-containing granules of the mouse juxtaglomerular cells.

Since the discovery that the juxtaglomerular cell (JGC) granules give a positive acid phosphatase reaction[1], the lysosomal nature of these granules has been repeatedly suggested[2-4]. In addition to the proteolytic enzyme renin, another lysosomal enzyme, β-glucuronidase was localized in the JGC granules[4]. The protein, carbohydrate and lipid histochemistry and vital staining characteristics of the JGC granules is very similar to those of the lysosomes[5-8], although differences have also been observed[6,9]. The aim of our present work was to obtain further information about the enzyme histochemistry of the renin granules with special respect to the lysosomal enzymes.

Material and methods. White female mice of our strain, weighing about 25 g and receiving a normal standard diet and water ad libitum, were used. The animals were killed under a light ether anaesthesia, the left kidney was immediately removed and subjected to the following histochemical procedures: 1. Light and electron microscopical aryl sulphatase reaction using p-nitro-catechol sulphate as substrate[10]. The time of fixation was 3 h and the pH of the incubation mixture was 5.4. For the electron microscopical investigation, 40 micra frozen sections were incubated and embedded into Epon. The fine sections were examined with and without contrast staining in a Tesla BS 513 A electron microscope.

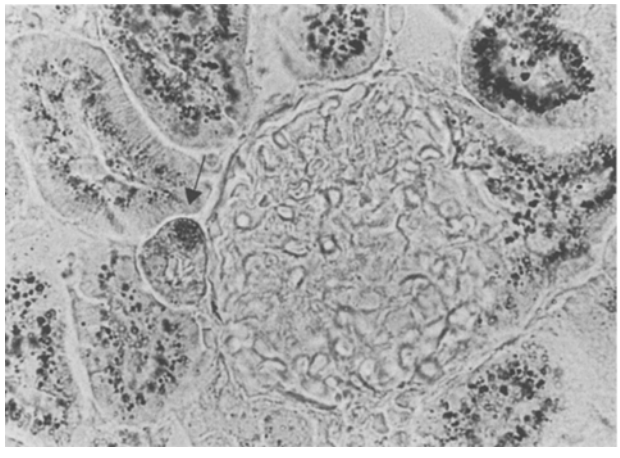
2. N-acetyl-β-glucosaminidase reaction for light microscopy on fixed material[11]. The blocks were impregnated with gum-sucrose before sectioning.

3. Nonspecific esterase using α-naphthyl acetate, naphthol AS-D acetate[12] or naphthol AS-D chloroacetate[13] as substrate. Fixation: a) Cold Baker calcium formol for 20 h. b) 3% glutaraldehyde in 0.2 M sodium cacodylate, pH 7.2. c) Freeze dried unfixed cryostat sections. The reactions were carried out for light microscopical investigation.

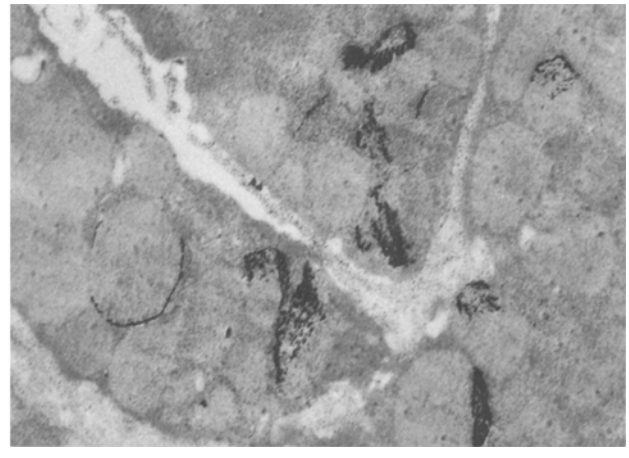
4. α-hydroxy acid oxydase reaction for peroxysomes[14].

5. Electron microscopical peroxydase reaction[15].

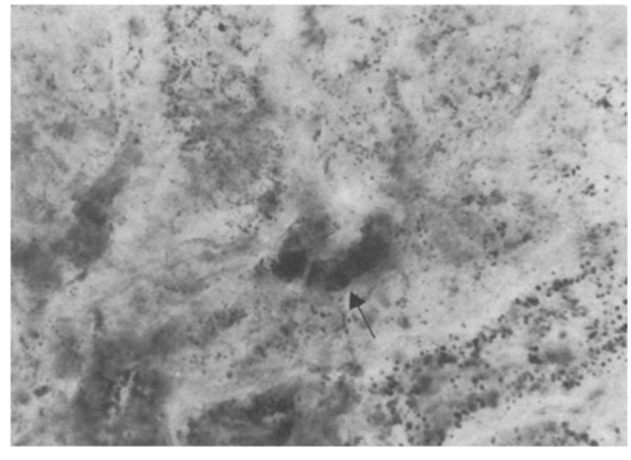
Observations and discussion. From the above-mentioned enzyme histochemical methods, only the aryl sulphatase and the N-acetyl-β-glucosaminidase reaction gave a positive result in the JGC granules. The aryl sulphatase gave a relatively weak light microscopical reaction, seemingly not every granule reacted within the same cell (figure 1). This impression was clearly justified with the electron microscope. A varying number of granules reacted in the cells and



1



2



3

Fig. 1. Light microscopic aryl sulphatase reaction. The arrow shows a cross sectioned afferent arteriole with granulated juxtaglomerular cells. Some of the granules give reaction. × 400.

Fig. 2. Electron microscopic aryl sulphatase reaction. Uncontrasted section. Some of the JG granules display reaction product, distributed unevenly at their periphery or in the matrix. × 18,000.

Fig. 3. Light microscopic Na-acetyl-β-glucosaminidase reaction. The arrow points to a heavily granulated juxtaglomerular cell group with an intense enzyme reaction. × 400.

the distribution of the reaction product was uneven in the matrix of the individual granules (figure 2).
The light microscopical N-acetyl-β-glucosaminidase reaction was stronger, apparently all the granules reacted, the JG cells rich in granules gave an almost diffuse reaction (figure 3). Electron microscopical localization of this enzyme has not been carried out. The nonspecific esterase, α-hydroxy acid oxydase and peroxydase reactions gave negative results in the JG cells.
Thus, in addition to the already known protease (renin), acid phosphatase and β-glucorinidase, 2 additional lysosomal enzymes, aryl sulphatase and N-acetyl-β-glucosaminidase, were demonstrated in the JGC granules.

Inspite of the close histochemical similarity between JGC granules and lysosomes, it has not been possible to demonstrate a functional similarity between them. After the administration of sucrose, iron sorbital-citric acid complex, horseradish peroxydase or thorium dioxide to the experimental animals, none of these substances was found to enter the JGC granules[16]. These functional differences do not exclude the possibility, that the ancestors of the JGC granules are lysosomes and that the lysosomal enzymes may have a role in the still incompletely understood mechanism of renin release.

1 J.H.C. Ruyter, Histochemie *3*, 521 (1964).
2 E.R. Fisher, Science *152*, 1752 (1966).
3 J.C. Lee, S. Hurley and J. Hopper, Lab. Invest. *15*, 1459 (1966).
4 Sz. Gomba and B.M. Soltész, Experientia *25*, 513 (1969).
5 Z. Nemes, E. Bodolay and Sz. Gomba, Acta Histochem. *55*, 80 (1976).
6 M. Cantin, Y. Desormeaux, J. Chlebovicova, S. Benchimol and M. de F. Araujo-Nascimento, Lab. Invest. *33*, 648 (1975).
7 V. Szokoly, Sz. Gomba and B.M. Soltész, Nature *203*, 1311 (1965).
8 K.A. Rosenbauer, Ergebn. allg. Path. path. Anat. *46*, 81 (1965).
9 Y. Desormeaux and M. Cantin, J. Microsc. Biol. cell. *27*, 37 (1976).
10 J.L.E. Ericson and H.J. Helminen, Histochemie *9*, 170 (1967).
11 M. Hayashi, J. Histochem. Cytochem. *13*, 355 (1965).
12 A.G.E. Pearse, Histochemistry: Theoretical and Applied. Churchill Livingstone, Edinburgh, London 1972.
13 L.T. Yam, C.Y. Li and W.H. Crosby, Am. J. clin. Path. *55*, 283 (1971).
14 T.K. Shnitka and G.G. Talibi, Histochemie *27*, 137 (1971).
15 R.C. Graham and J.M. Karnovsky, J. Histochem. Cytochem. *14*, 291 (1966).
16 M. Cantin, Y. Desormeaux and S. Benchimol, Beitr. Path. *161*, 310 (1977).

Ultrastructural study of somatotroph cells from mice bearing a fast growing transplanted hepatoma, in different periods of the tumor development

Marta Souto, F.R. Moreno and C.L.A. Gómez Dumm[1]

Instituto de Embriología, Biología e Histología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Calle 60 y 120, La Plata (Argentina), 17 July 1978

Summary. The presence of a transplanted, fast-growing hepatoma (SS1-K) produces conspicuous ultrastructural changes in pituitary STH cells of C3H-S male mice. These changes are suggestive of an increased secretion of growth hormone only during the first stages of the tumor development. The hepatoma influence does not seem to be clearly related to the illumination regimen or time of killing.

Badrán et al.[2] have studied the ultrastructure of the pars distalis of the pituitary in mice bearing transplanted hepatomas. Their observations revealed changes in somatotroph (STH) cells suggesting an enhancement of their secretory activity. Besides, the fine structure of adenohypophysis from rats with transplanted tumors was found to present variations, depending on the degree of the tumor growth[3].
Recently, we have reported circadian variations in STH cells of mice bearing a slow-growing transplanted hepatoma[4]. The present paper attempts to analyze the electron microscopy of STH cells in the same strain of mice after transplantation of a fast-growing hepatoma (SS1-K), studying the pituitary at 3 time-points of different stages of the tumor development.
Material and methods. C3H-S male mice were used for the experiment. The animals were kept under standard conditions for periodicity analysis[5] and single-caged at a temperature of 25 °C, with water and food ad libitum. They were illuminated from 06.00 to 18.00 h, alternating with 12 h darkness.
An SS1-K Wilson hepatoma was transplanted in the interscapular region of 45 normal 4-week-old male mice. The SS1-K hepatoma is fast growing and reaches a mean diameter of about 2 cm in 30 days. This tumor kills 50% of the mice in 40 days, and it can be considered a poorly differentiated carcinoma. The biological behavior and histomorphology of SS1-K hepatoma has already been studied in our laboratory[6].
The 45 mice were separated into groups of 5 animals each. Normal intact mice were used as controls for each group. The animals were killed 13 (group I), 27 (group II) and 43 days (group III) after transplantation. The mean weights of the tumors were 0.137 g, 1.039 g and 11.882 g, respectively. The mice were sacrificed at 00.00, 12.00 and 16.00 h in each different period of the tumor growing, taking into account previous data about time variations[4]. The animals were killed by decapitation and exanguination, the pituitaries were removed and their lateral wings were separated and sliced into small pieces. The material was fixed 90 min in 1% osmium tetroxide according to Millonig[7], dehydrated in ethanol and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop I electron microscope.
Results. The STH cells were easily identified in the pars distalis and presented the general aspect previously reported in mice[8,9]. The presence of the SS1-K hepatoma produced conspicuous changes in the fine structure of these cells. The rough endoplasmic reticulum appeared more extended, irregular and dilated in nearly all the animals, at different times (figure 1). The changes were found in the