Histochemistry of Lysosomal Enzymes in Juxtaglomerular Cells

Recent light and electron microscopic histochemical investigations detected acid phosphatase activity in the juxtaglomerular cell (JGC) granules of the mouse and rat (RUYTER[1], LEE, HURLEY and HOPPER[2-4], GOMBA, SOLTÉSZ and SZOKOLY[5]). The same observation was made on human (FISHER, PEREZ-STABLE and PARDO[6]) and on rhesus monkey (ROSEN and TISHER[7]) JGC granules. OGINO, MATSUNAGA, SAITO, KIRA, TAKAYASU and ONO[8] localized renin and acid ATP-ase in the lysosome fraction of renal subcellular particles isolated by differential centrifugation and considered both of them to be lysosomal enzymes. According to FISHER[9], the JGC granules have a lysosomal character represented by their acid phosphatase and proteolytic (renin) activity. We have not been able to find data in the literature as to whether these granules contain any other lysosomal enzymes. In the present investigation we have studied the histochemical activity of 4 lysosomal enzymes in the mouse JGC.

White mice of our own strain of both sexes weighing about 20 g were narcotized with ether and the kidneys were fixed in situ by injecting cold Baker calcium formol into the aorta via the left heart ventricle for about 3 min. The left renal vein was incised before starting with the injection. Then the left kidney was immediately removed and immersed in cold gum-sucrose (Holt) for 24 h. The following enzyme histochemical methods were accomplished, using unmounted 8 μ cryostat sections: (1) Naphthol AS-BI phosphate, freshly diazotized Edelrotamine ITR (N', N' diethyl-4-methoxy sulphanyl amide) method for acid phosphatase. Concentration of the substrate was 1.04 mM, concentration of the coupling agent 5 mM, in 0.05 M acetate buffer pH 5.2. (2) HAYASHI, NAKAJIMA and FISHMAN's[10] method for β-glucuronidase using naphthol AS-BI β-D-glucuronide as a substrate. The coupling agent was again the freshly diazotized Edelrotamine ITR, its final concentration in the incubation mixture being 2.5 mM. (3) Methods for aspecific esterase. (A) Indoxyl acetate procedure (BARKA and ANDERSON[11]). (B) Naphthol AS-D acetate method (BARKA and ANDERSON[11]). The reaction was carried out on pH 7.0 or 5.0 using fast blue RR as a coupling agent. (4) RUTENBURG, COHEN and SELIGMAN's[12] method for aryl sulphatase in our modification. The original post-coupling method was transformed into a simultaneous one. The concentrations of the components in the incubation mixture were the following: substrate 1 mg/ml, fast blue RR 0.5 mg/ml, in 0.01 M acetate buffer pH 6.1.

The tubular lysosomal elements reacted positively in all of the incubation mixtures mentioned, but only the acid phosphatase and β-glucuronidase methods gave a positive result in the JGC granules. The activity of the β-glucuronidase was more intense than that of the acid phosphatase. No staining was present in sections incubated without substrate. Very few weakly reacting lysosomes were detected with the methods mentioned also in the renal arterial as well as arteriolar smooth muscle cells.

The presence of 2 typical lysosomal enzymes in the JGC granules gives a further support to the concept of their lysosomal nature. Besides the well-known fact that secretion vacuoles in different tissues show one or more characteristics of the lysosomes, the lysosomal character of the JGC granules has a special importance. It suggests that the transformation of the vascular smooth muscle cells into epithelial-like endocrin elements is probably based on the activation of their lysosomal system, this being rather inactive in normal, unmodified arteriolar smooth muscle cells. During this activation the production of some lysosomal enzymes (nonspecific-esterase, arylsulphatase) diminishes, and that of others (acid phosphatase, β-glucuronidase and first of all the proteolytic renin) increases. According to the lysosomal concept of the secretory activity of JGC, the smooth muscle cell-secretory gland cell transformation would be generated by the redifferentiation and specialization of an old defending-digesting system of the cell. Nevertheless, for the time being this is not more than a hypothesis, which needs further investigation.

Zusammenfassung. In den Granula juxtaglomerulärer granulierter Mäusenierenzellen wurde eine saure Phosphatase und eine β-Glykuronidase-Aktivität nachgewiesen. (Methoden für Arylsulphatase und unspezifische Esterase waren negativ.) Es wird der Zusammenhang mit der Lysosomenart der Granula diskutiert.

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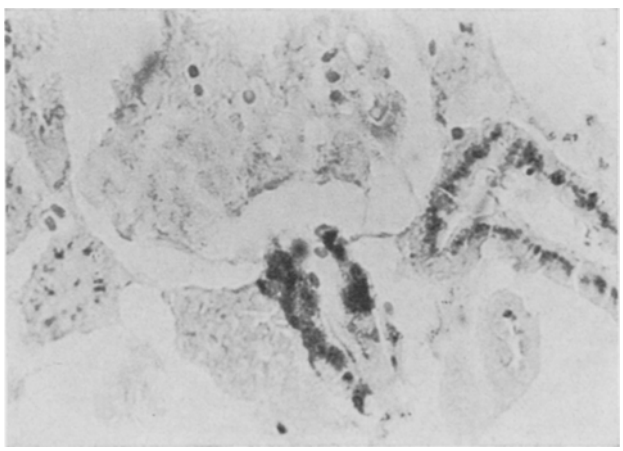

β-glucuronidase activity in juxtaglomerular cell granules in the wall of an afferent arteriole of the mouse kidney. × 400.

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