Organization of the Sympathetic Innervation in Liver Tissue from Monkey and Man


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Summary. The sympathetic innervation of the liver of monkey and man has been investigated in a combined fluorescence histochemical, chemical and electron microscopical study. By means of the Falck-Hillarp fluorescence method a dense network of monoamine-containing nerve fibers was visualized in liver tissue of monkey and man. The nerve fibers ran in close contact to both hepatocytes and blood vessels. Chemical quantitations showed high concentrations of noradrenaline in both human and monkey liver. Microspectrofluorometry of the intraneuronal monoamine resulted in spectra characteristic of a catecholamine. For the electron microscopical study the dopamine analogue, 5-hydroxydopamine, was used to “label” the catecholamine terminals in both human and monkey liver. The nerve profiles, identified as catecholamine-containing, were demonstrated in a perivascular location and in close contact to hepatocytes. No synaptic membrane specializations were present between nerve fibers and hepatocytes. The general ultramorphology and intralobular distribution pattern of nerves in the liver of monkey and man were similar. The present results prove the existence of a sympathetic innervation of hepatocytes and blood vessels in the liver of man and monkey.

Key words: Liver (man, monkey) – Sympathetic innervation – Catecholamine fluorescence – Adrenergic mechanisms.

The organization and function of the autonomic nervous system in liver tissue have long been the subject of intense research (Riegele, 1928; Ungvary and Donath, 1969; Edwards, 1971, 1972; Skaaring and Bierring, 1976). Experiments on calves, dogs and cats have shown a considerable influence of sympathetic stimulation on liver function. Thus, electrical stimulation of the liver branches of the splanchnic nerve was associated with a variation in the rate at which glucose was released from the liver (Edwards, 1971, 1972; Järhult, 1975). Indications for functional
importance of a parasympathetic innervation was presented by Black and Reis (1971), who demonstrated that vagal stimulation resulted in significant elevation of hepatic tyrosine transaminase activity. Furthermore, Shimazu and Fujimoto (1971) showed that incorporation of radioactive glucose into liver glycogen was markedly increased following vagal stimulation. The existence of autonomic nerves innervating liver parenchyma could therefore be expected. Ungvary and Donath (1969) analyzed the distribution of adrenergic nerves in the liver of a series of laboratory animals; they found adrenergic nerves only in relation to blood vessels. By using techniques for the demonstration of cholinesterase activity presumed cholinergic fibers have been demonstrated in relation to blood vessels and hepatocytes in monkey, guinea pig and rat liver (Sutherland, 1964; Skaaring and Bierring, 1976).

Ultrastructural studies on nerves in the liver parenchyma have shown nerve profiles with dense core vesicles intralobularly in mice (Yamada, 1965) and in the space of Disse, and in relation to hepatocytes and Kupffer cells of the tree shrew (Forssmann and Ito, 1977).

This study reports on a sympathetic innervation of liver parenchymal cells in monkey and man.

Materials and Methods

The human liver specimens were obtained from biopsies taken during operations. All specimens were examined with routine histological stainings and only liver tissue without pathological changes was included. Monkey liver tissue was obtained from five rhesus monkeys (Macaca mulatta). The animals were killed by bleeding during barbiturate anesthesia.

Fluorescence Microscopy and Microspectrofluorometry. Within 10 min after the liver pieces had been removed, they were frozen in a propane-propylene mixture at the temperature of liquid nitrogen. After freeze-drying the preparations were processed for the fluorescence microscopical visualization of biogenic monoamines according to the Falck-Hillarp method (Falck et al., 1962; Falck, 1962; for technical details, see Björklund et al., 1972). The sections were mounted either on microscope slides for fluorescence microscopy or on cover slips for microspectrofluorometric analysis.

The microspectrofluorometric analysis was performed with a modified Leitz microspectrofluorometer according to Björklund et al. (1972).

Electron Microscopy. For ultrastructural investigation, the biopsy specimens of human liver were cut into thin blocks and incubated in buffer at 37°C containing 10⁻⁶ M 5-hydroxydopamine (5-OH-DA) for 45 min. Control samples were incubated without 5-OH-DA. After termination of the incubation the specimens were fixed in cold glutaraldehyde (3%) dissolved in phosphate buffer, pH 7.3, for 2 h, rinsed in buffer and postfixed in 1% Os₂O₄ dissolved in phosphate buffer containing 0.1 M sucrose. They were then dehydrated in alcohol/propylene oxide and embedded in Epon 812. Three adult rhesus monkeys (raised at the Heinrich-Pette-Institut, University of Hamburg) were perfused (in sodiumpentobarbitone anesthesia, 40 mg/kg body weight) with 6% glutaraldehyde (dissolved in 0.05 M phosphate buffer) via the heart, 30 min after intravenous injection of 50 mg/kg 5-OH-DA. 15 min after the onset of glutaraldehyde perfusion, small pieces of tissue were removed from the liver and postfixed in 1% Os₂O₄ dissolved in phosphate buffer containing 0.1 M sucrose. After rinsing in buffer solution, the specimens were dehydrated and embedded. Semithin and ultrathin sections were cut on a Reichert ultramicrotome Om U2 and contrasted with lead citrate prior to electron microscopical analysis in a Philips EM 300 electron microscope.

Noradrenaline Determinations. Noradrenaline (NA) in liver tissue was determined fluorometrically according to the method of Bertler et al. (1958) and Häggendal (1963).

# For preliminary reports see Falck et al. (1975) and Nobin et al. (1977)