Demonstration of Noradrenaline-Immunoreactive Nerve Fibres in the Liver

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To demonstrate noradrenaline-immunoreactive nerve fibres in liver tissues, we used an antibody to noradrenaline in the immunostaining of liver tissues from rats, guinea-pigs and humans. The tissue specimens were fixed by perfusion or immersion with cacodylate buffer containing sodium metabisulphate and glutaraldehyde, and cryostat sections were prepared. An indirect peroxidase-labelled antibody method was used for staining noradrenaline. Noradrenaline-immunoreactive nerve fibres were localized around blood vessels in the portal area and around the central vein. There were differences between the species in the intralobular distribution of noradrenaline-immunoreactive fibres. Normal guinea-pig and human liver showed intralobular noradrenaline-immunoreactive fibres while rat liver did not. Noradrenaline-immunoreactive fibres were absent from regenerating nodules in a human cirrhotic liver. This method of demonstrating noradrenaline directly using perfusion- or immersion-fixation is appropriate for studying innervation in normal and damaged livers of various species including humans.

KEY WORDS: Noradrenaline; Catecholamine; Adrenergic nerve fibre; Anti-noradrenaline antibody; Autonomic nervous system; Hepatic innervation; Immunocytochemistry; Liver
INTRODUCTION

The liver is innervated by branches of the autonomic nervous system and the distribution of nerve fibres in the liver has been reviewed.1 To detect nerve fibres in liver tissues, various histochemical or immunocytochemical markers have been used. S100, neuron-specific enolase,2 PGP9.5,3 synaptophysin,4 and neural-cell adhesion molecules5 are useful for mapping the total innervation of the liver. Fluorescence histochemistry6-8 or histochemistry for cholinesterase9 have been used to delineate adrenergic or cholinergic innervation of the liver. Recently, neuropeptides and catecholamine-synthesizing enzymes have been used to study the distribution of nerve fibres with various neurotransmitters.10-15 The detection and localization of noradrenaline (NA), one of the classical neurotransmitters, in liver tissues has, however, been generally neglected.16

In the present study, we have used an antibody to NA to demonstrate directly and specifically NA-immunoreactive (IR) nerve fibres in liver tissues.

MATERIALS AND METHODS

TISSUE SPECIMENS

The four solutions listed in Table 1 were prepared for tissue fixation.17 Tissue specimens were fixed according to the method of Geffard et al.17,18 with some minor modifications.

RAT AND GUINEA-PIG LIVER

Male Wistar rats (n = 4) and male Hartley guinea-pigs (n = 4) were anaesthetized with pentobarbital, and perfused with solution A for 30 s to wash out the blood, and with solution B for 3 min.17 The livers were removed, cut into 1 mm slices, post-fixed with solution C for 6 h and washed in solution D for 6 h. The fixed specimens were embedded in Tissue-Tek OCT compound (Miles Pharmaceutical, Naperville, IL, USA), frozen in dry ice–ethanol, and sectioned at 10 μm thickness.

HUMAN LIVER

Six specimens of human liver were used. Informed consent was obtained from all the patients. Four specimens were obtained at hepatectomy for hepatocellular carcinoma, haemangioma of the liver and liver tumour which was subsequently diagnosed as focal nodular hyperplasia. Non-tumourous areas of these livers were used in the study and their histology showed liver cirrhosis (two cases) or normal liver (two cases). The other two liver specimens were obtained at cholecystectomy or cholecystolithiasis in order to

| TABLE 1 |
|---|---|---|---|
| **Solutions used in the fixation of liver tissue specimens** | | | |
| **Solution** | **Buffer** | **Sodium metabisulphate concentration (%)** | **Glutaraldehyde concentration (%)** | **pH** |
| A | Cacodylate (0.1 M) | 1 | — | 6.2 |
| B | Cacodylate (0.1 M) | 1 | 5 | 7.5 |
| C | Cacodylate (0.1 M) | 1 | 2.5 | 7.7 |
| D | Tris (0.005 M) | 0.85 | — | 7.5 |
evaluate abnormal liver-function tests; they were subsequently found to be fatty or normal liver.

The specimens were dipped into solution A, cut into 1 mm slices, immersed in solution C for 6 h, and then washed in solution D for 6 h. The fixed specimens were embedded and sectioned by the same method as the experimental animal liver specimens.

**ANTIBODIES**
Anti-NA antiserum raised in rabbits was obtained from Société Francaise de Recherches et d’Investissements Laboratoire (SFRI) (Cosmo Bio, Tokyo, Japan). The antibody was diluted to 1:1200 with solution D containing 2% bovine serum albumin. Peroxidase-conjugated F(ab’)_2 fragments of goat antiserum to rabbit IgG were purchased from Cappel (Cosmo Bio, Tokyo, Japan).

**IMMUNOCYTOCHEMISTRY**
The sections were reduced with solution D containing 0.1 M sodium borohydride for 10 min to reduce the double bonds between glutaraldehyde and NA or tissue protein. The indirect peroxidase-labelled method was used for immunocytochemistry. This method involves successive incubation with anti-NA antiserum for 16 h at 4°C. After the incubation, the sections were treated with methanol containing 0.1% hydrogen peroxide for 20 min to inactivate endogenous peroxidase. The sections were incubated with the second antibody for 16 h at 4°C and then dipped in 0.025% diaminobenzidine solution containing 10 mM hydrogen peroxide and sodium azide for 10 min, and counter-stained with methyl green.

**CONTROL STAINING**
The specificity of the staining was confirmed by the use of non-immune rabbit serum instead of the primary antiserum.

**RESULTS**
The results are summarized in Table 2.

**RAT LIVER**
Noradrenaline-immunoreactive fibres were detected in the portal tract; they were mainly in close contact with the hepatic artery and the portal vein. Nerve fibres were not detected in the hepatic lobule (Fig. 1) except in the lobule adjacent to the portal area and around the central vein.

**GUINEA-PIG LIVER**
Noradrenaline-immunoreactive fibres were localized in the portal tract and around the vasculature. They were also localized within the hepatic lobule (Fig. 2), along the sinusoid and around the central vein.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Area</th>
<th>Rat liver</th>
<th>Guinea-pig liver</th>
<th>Human liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal or</td>
<td>fatty liver</td>
<td>Cirrhotic</td>
</tr>
<tr>
<td>Portal area</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lobule</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Central vein</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*, fibres detected; -, fibres not detected.

* A few nerve fibres were detected in the lobule adjacent to the portal area.
Rat liver. Noradrenaline-immunoreactive nerve fibres are detected around the blood vessels in the portal area but not in the hepatic lobule (magnification x 215).

Guinea-pig liver. Noradrenaline-immunoreactive nerve fibres are detected around the blood vessels in the portal area. Noradrenaline-immunoreactive fibres are also stained granularly along the sinusoid of the hepatic lobule (arrow heads) (magnification x 215).
Noradrenaline-immunoreactive nerve fibres in the liver

**FIGURE 3**

*Human fatty liver. Noradrenaline-immunoreactive nerve fibres are detected along the sinusoid of the hepatic lobule (magnification x 215).*

**HUMAN LIVER**

Noradrenaline-immunoreactive nerve fibres were localized in the portal area. Nerve fibres were detected in close contact with the blood vessels in all normal and diseased liver. They were also demonstrated along the sinusoid of the hepatic lobule (Fig. 3) except in the case of the patient with cirrhosis in whom NA-IR fibres were absent from regenerating nodules.

**CONTROL STAINING**

No reaction product was detected in the control section.

**DISCUSSION**

The present study demonstrated NA-IR nerve fibres in the liver tissues of rats, guinea-pigs and humans, using an antibody to NA.

There are species differences in hepatic innervation. In the mouse and rat, nerve fibres are distributed only in the portal area of the liver and are mainly localized around the blood vessels. In humans, primates and guinea-pigs, the liver receives not only portal- but also intralobular innervation. Adrenergic nerve fibres are distributed among hepatocytes and sinusoidal lining cells within hepatic acini. The disappearance of intralobular innervation in human cirrhotic liver has been reported previously and the present results were consistent with this.

Some aspects of the methods used in this study are noteworthy. The antibody was raised by immunization using an NA–glutaraldehyde–carrier protein conjugate and reacts with NA–glutaraldehyde–carrier protein,
but not with NA or dopamine alone. The tissue specimens must, therefore, be fixed using the solutions containing glutaraldehyde listed in Table 1. Furthermore, the bonds between NA and glutaraldehyde and between glutaraldehyde and tissue protein must be single bonds. The cross-reactivity of the antibody with NA-glutaraldehyde conjugates containing double bonds is very low. 17

The original methods developed by Geffard et al. 17,18 involved perfusion fixation. We applied the same methods not only to fixation by perfusion in experimental animals but to immersion fixation of human liver tissues. In both cases tissue fixation and immunostaining were satisfactory, indicating that the present methods might be useful tools for studying the distribution of noradrenergic innervation in human tissues.

REFERENCES


12 Fehér E, Fodor M, Fehér J: Ultrastructural localization of somatostatin- and substance P-immunoreactive nerve fibres in


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