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Immunohistochemical evidence for mast cells containing 5-hydroxytryptamine in the rat portal vein

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Abstract

Serotonin was localized to mast cells in the adventitia of the rat portal vein by indirect immunohistochemistry. The mastocytes where preferentially localized to a region delimited by the pyloric and splenic veins. Since neither 48/80 nor reaginic antibody induced a significant change in the intrinsic spontaneous activity of the portal vein it would appear that the mast cells are not involved in a direct vasomotor function. It is suggested that amines released from the mastocytes could regulate blood flow in the vasa vasorum and/or have a role associated with the sensory functions displayed by this vein.

Introduction

Several conflicting reports exist concerning the presence of mast cells around rat blood vessels. Very few mast cells were found around the vascular tissue examined by EL-AKARD and BRODY [1], although RILEY [2], showed the presence of mast cells in the tunica adventitia of all of the vessel examined.

In the majority of species histamine is the predominant amine found in mast cells, although in rats and mice both histamine and 5-hydroxytryptamine (5-HT) have been identified in these cells [3, 4]. Based on the yellow fluorescence, typical for 5-HT when it is revealed by the condensation reaction of FALCK *et al.* [5], BOOZ [6] suggested that the mast cells surrounding the rat portal vein contain 5-HT. In this study we confirmed the observation of Booz, by using the indirect immunohistochemical staining method of COONS [7]. In addition, we examined the portal vein for the coexistence of substance P and 5-HT in nerve fibres or mast cells, since several reports suggest the coexistence of these two substances in rat CNS and other areas [8, 9].

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Materials and methods

Histochemistry

Male and female Sprague-Dawley rats weighing 200–250 g were used. The portal veins were perfused *in situ* with 4% formaldehyde in 0.1 *M* phosphate buffer (PBS; pH 7.2–7.4), removed from the animal and immersed in the same fixative for 6–8 h and then washed overnight in 5% sucrose. The veins were then sectioned (10–15 μm) and mounted on slides for processing with the indirect immunohistochemical technique of COONS [7]. The tissues were incubated overnight at room temperature with either a 1:400 dilution of serotonin antiserum [8] or a 1:300 dilution of a substance P antiserum [10]. Following a rinse in PBS the tissues were incubated with fluorescein-isothiocyanate of 1:100. After a final rinse the tissues were mounted in glycerol-PBS (1:3), and examined under the Leitz Orthoplan microscope with filter-mirror system I_2. Sections incubated with the antisera preabsorbed with either 5-HT, or substance P, at 10^{-5} *M* served as controls.

The method of FALCK *et al.* [5] was used to demonstrate the amines in the mast cells. Whole mounts preparations (spread) and tissue sections were dried in a dessicator for 3 h over P_2O_5, and then subsequently treated with paraformaldehyde vapour at 85°C for 2 h. The tissues were examined with a Leitz-Orthopian microscope with a filter-mirror system A.

Following fluorescence microscopy the tissues were stained for histological revelation of the mast cells. The tissues were stained with toluidene blue (0.8%) in 40% ethanol for 10 min for the whole mounts and for 1 to 3 min for the sections. After washes in 80% ethanol (2 × 10 min), a rinse in absolute ethanol, a clearing in xylol, the tissues were mounted in antellan.

Denervation

One group of rats were injected i.p. over two days with

2 × 50 mg/kg of 6-hydroxydopamine (6-OHDA) and another group of animals were treated with reserpine (2 × 5 mg/kg), according to described procedures [11]. Both 6-OHDA and reserpine were dissolved in 0.9% NaCl containing 1 mg/l of ascorbic acid. Control animals were injected with the vehicle.

Immunization

Rats were sensitized by intraperitoneal injections of 10 μg of ovalbumin suspended in $Al(OH)_3$ as adjuvant [12]. A booster injection with adjuvant was given ten days after the first injection and the portal veins were examined for an anaphylactic response on days 21 and 22.

Vasomotor actions of 5-HT, histamine and 48/80 [13]

Portal veins, prepared as longitudinal strips, were examined for the spasmogenic actions of 5-HT, histamine and 48/80. In addition, the vasomotor responses of portal veins obtained from sensitized animals to an ovalbumin challenge were determined. The portal veins were mounted in a microchamber [13] under 500 mg of tension for measurement of isometric contractions and continuously perfused at 35° C with a Krebs solution of the following composition: NaCl 122 m*M*; KCl 4.7 m*M*; $CaCl_2$ 2.8 m*M*; KH_2PO_4 1.2 m*M*; $NaHCO_3$ 24.8 m*M*; $MgSO_4$ 1.2 m*M*; glucose 10.1 m*M*; 95% O_2/5% CO_2; pH 7.4, 5-HT, Histamine and 48/80 were added to the perfusate to obtain the desired final concentration.

Materials

All standard chemicals used in this study were obtained from commercial sources. The substance P antiserum was produced in our laboratory and it has been extensively characterized [10]. The serotonin antiserum was generously provided by Dr Steinbush and its specificity has been described [8].

Results

With spread preparations of the rat portal vein, treated by the Falck-Hillarp method, numerous cells as well as sympathetic nerves were visible in the adventitia (Fig. 1A). The yellow fluorescence of these cells contrasts with the green fluorescence of the nerves. The fluorescent cells were frequently observed near small blood vessels (Fig. 1B). The cells were found exclusively in the segment of the portal vein delimited by the pyloric and splenic veins, with the major portion being near the pyrolic vein. When transverse sections were examined with both paraformaldehyde condensation (Fig. 2A) and the serotonin antibody (Fig. 2C) techniques fluorescent cells were found in the adventitia and at the adventitial-media junction, whereas no labelled cells were seen in the media or in the intima. Since these cells stained metachromatically with toluidine blue (Fig. 2B and 2D) they are undoubtedly mast cells.

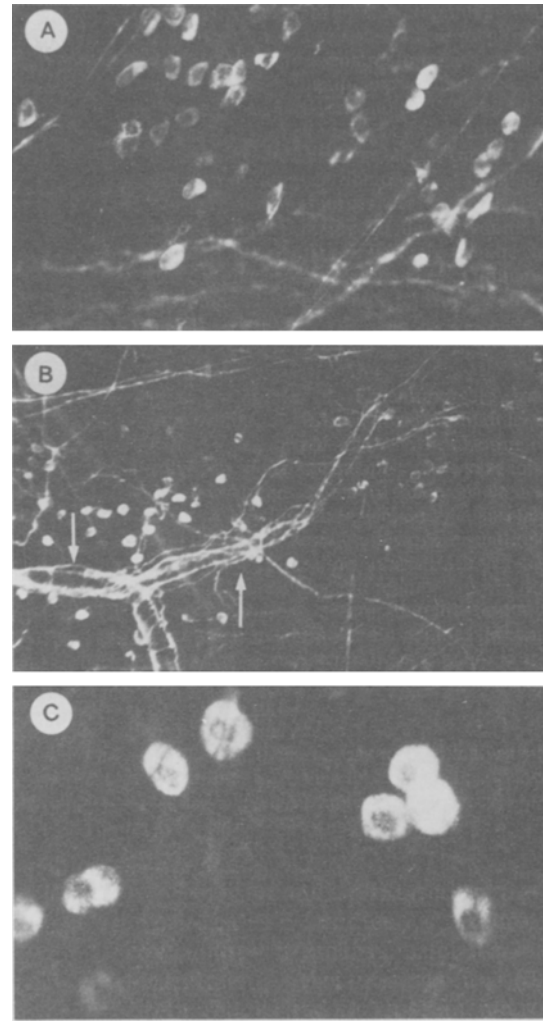

Figure 1
Fluorescence of amines in mast cells and nerve fibres in spread-preparations of the rat portal vein that were treated with the Falck-Hillarp method.

A. The mast cells and nerve fibres in adventitia, × 224.
B. Numerous mast cells surround a small blood vessel (arrow) in the adventitia, × 147.
C. Fluorescent mast cells in whole-mount preparation of portal vein after pretreatment with 6-hydroxydopamine.

Pretreatment of the animals with 6-OHDA resulted in a depletion of the catecholamines from the nerve plexuses found in the portal vein, but fluorescence in the mast cells were not affected (Fig. 1C). In contrast reserpine

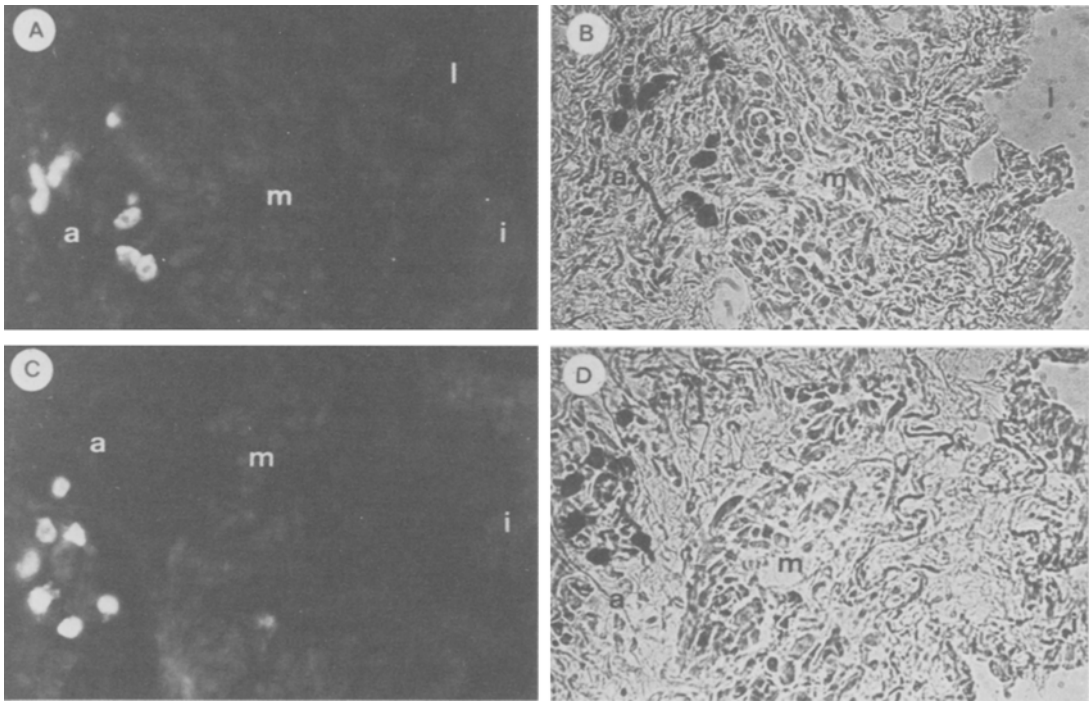

Figure 2
Mast cells in transversal sections of the portal vein (a = adventitia, m = media, l = luminal).
A. Fluorescent mast cells after formaldehyde treatment (Falck-Hillarp method).
B. Same section stained with toluidine blue.
C. Immunohistochemical demonstration of serotonin in mast cells at the adventiatial-medial border of portal vein.
D. The same section shows a mast cell stained metachromatically with toluidine blue × 280.

abolished all fluorescence from the mast cells and the sympathetic nerves.

A network of the substance P-containing nerve fibres were found in the portal vein, but 5-HT was never observed in nerve fibres (results not shown). In addition, a co-localization of substance P and 5-HT in the mast cells was never observed.

Upon addition of 48/80 to the isolated rat portal vein no change in the spontaneous myogenic activity was noted. 5-HT was capable of eliciting a pronounced venoconstruction ($ED_{50} = 5.35$) and histamine was much less potent ($ED_{50} = 3.30$). Portal veins obtained from ovalbumin sensitized rats when challenged with ovalbumin (10^{-5} g/ml or 10^{-3} g/ml) did not produce consistent or marked vasomotor response. Only four out of twelve tissues tested responded with a small increase in the frequency of the spontaneous contractions. All tissues, however, responded to exogenous acetylcholine (10^{-6} and 10^{-5} m) with a tetanic contraction. Furthermore, lung strips prepared from sensitized animals, as has been described previously [12], responded to the antigen challenge with a contractile response.

Discussion

The rat portal vein responds to a variety of neurotransmitters, hormones, and neuropeptides with a vasoconstriction [11, 13, 14]. Several of these vasoactive agents are localized in intramural structures; noradrenaline, acetylcholine and substance P are found in nerve fibres [6, 10, 11, 15] and now 5-HT has been positively identified in the mast cells. Since the vasoconstriction induced by transmural field stimulation of the portal vein can be totally abolished by phentolamine, noradrenaline is the primary

vasomotor neurotransmitteur [11]. Although the role of acetylcholine containing nerves is not known [15], substance P undoubtedly subserves a sensorial function [16, 17].

The role of 5-HT remains unknown but a vasomotor role can be excluded since neither compound 48/80, a potent stimulant of mast cell degranulation [18], nor reaginic antigen modified vascular tone. Although the type of mast cell located around the portal vein has not been identified definitively, they probably are several type cells, which will degranulate with a 48/80 stimulation [18].

Furthermore, isolated lung strips contracted upon addition of the antigen, proving that the mast cells had been sensitized. These observations suggest that the quantity of histamine available for release from the mast cells is not sufficient to activate the smooth muscle of the portal vein. Nevertheless the 5-HT receptors on the portal vein [14, 19] could respond to circulating 5-HT.

The functional role of the mast cells in the adventitia of the rat portal vein remains to be identified. The amines released from these cells not only may serve to regulate blood flow in the vasa vasorum of the portal vein but also may contribute to the sensory functions associated with this blood vessel. Osmoreceptors [20] and nocioceptors sensitive to bradykinin [16] are found in the portal vein. The substance P, cholinergic and sympathetic innervation of the portal vein as well as the mast cells may operate in an integrated system in the maintenance of visceral homeostasis.

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