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Serotonin immunoreactivity in the intermediate lobe of the rat pituitary

J.C. Carvajal[1], S. Carbajo[2], E. Carbajo-Pérez[2], S. Castro[1] and J. Rodríguez[1]

Department of Anatomy and Histology, University of Salamanca, Spain, [1]School of Medicine of Avila and [2]Faculty of Medicine of Salamanca, Salamanca, Spain

Summary. Immunocytochemical staining for serotonin (5-HT) in paraffin-embedded sections of rat pituitary resulted in the localization of reactive nerve fibres and cell bodies in the intermediate lobe. Immunostaining was also found in the anterior and posterior lobes. Labelled nerve fibres appear to enter the intermediate lobe from the neural lobe through the interlobular spaces. These fibres are relatively scarce and lightly stained. Neuroglandular contacts were identified between varicose nerve endings containing serotonin and immunoreactive perykarion. It is not clear whether intermediate lobe cells produced 5-HT themselves or, alternatively, these cells take in 5-HT from serotoninergic nerve terminals.

Key words: Serotonin, Immunocytochemistry, Pituitary, Intermediate lobe, Rat

Introduction

The role of dopamine (DA) and gammaaminobutyric acid (GABA) in the control of the pituitary intermediate lobe (IL) is well documented (Briaud et al., 1979; Demeneix et al., 1984; Taleb et al., 1986). Nerve fibres containing DA (Bjorklund et al., 1973). GABA (Rabhi et al., 1987; Carbajo et al., 1989), or both neurotransmitters simultaneously (Vuillez et al., 1987; Carbajo et al., 1987) have described in the IL.

In contrast, the serototinergic innervation of the IL is much disputed. Calas (1981) reported the existence of fibres which selectively take in ^{3}H-5HT in the IL of rats and mice and, shortly after, Schimchowitsch (1984) identified fibres reactive to a monoclonal antibody to 5-HT in the IL of rabbits. However, Kondo et al. (1983) could not demonstrate the serotoninergic innervation of IL in the rat, guinea pig and dog though the same authors showed the existence of serototinergic fibres in the IL of lower vertebrates, agreeing with the findings of Ueda et al. (1984).

Neither is the action of serotonin upon the IL clear. Perifused IL of rats show no reaction to the administration of 5-HT, whether or not cAMP is present (Baker, 1976; Tilders, 1979). The compound p-chlorophenylalanine, which inhibits the enzyme tryptophan hydroxilase has no effect on the concentration of MSH in the IL of mice (Francis and Barnawell, 1978). However, the MSH content of the IL decreases after the injection of 5-HT, this effect being blocked by metisergide (Taleisnick et al., 1973).

All in all, we though it would be worth reinvestigating the innervation of the rat IL using an antibody to 5-HT.

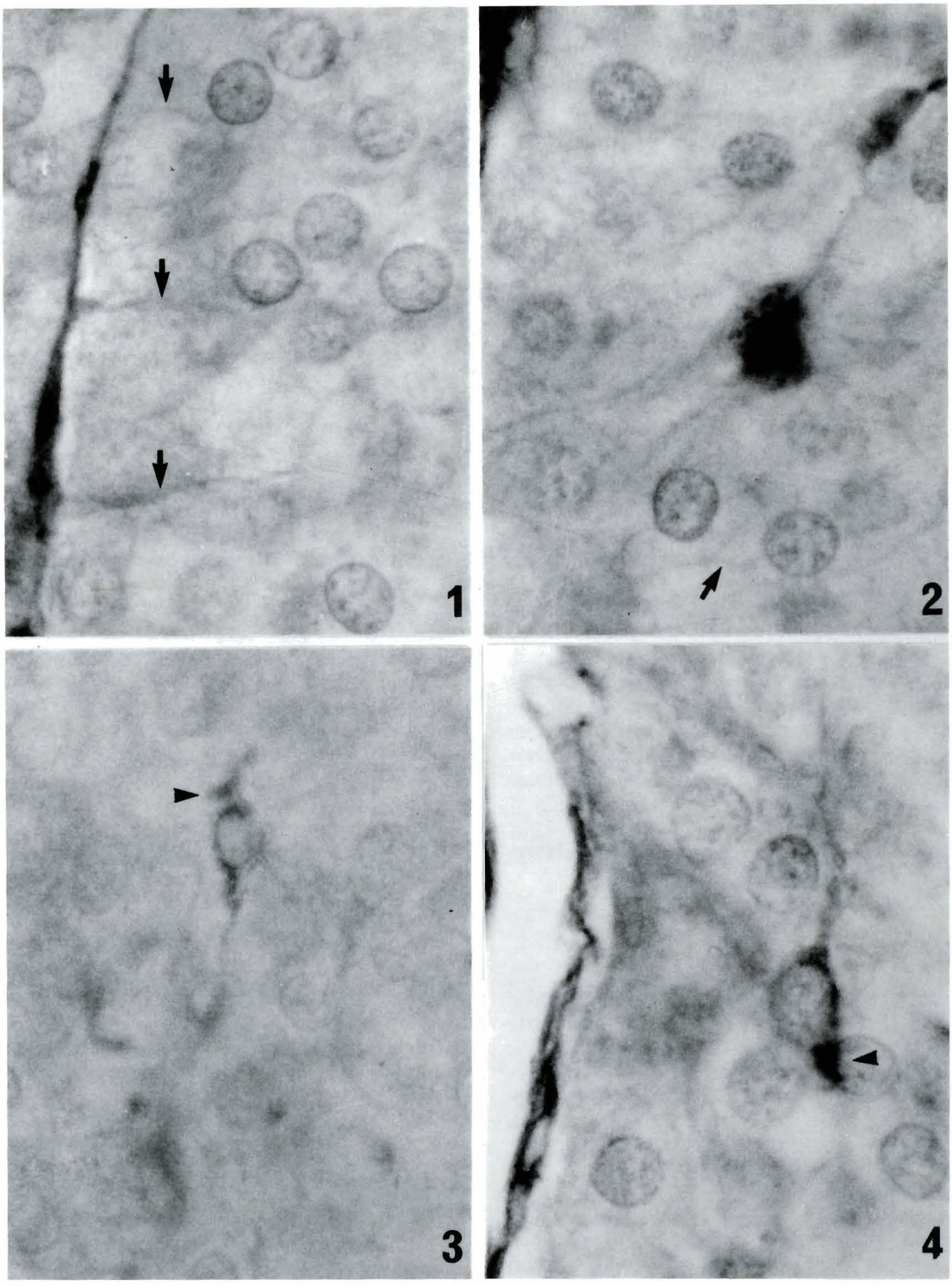
Materials and methods

Young adult Sprague-Dawley rats (200-220 g. body weight) were used throughout. After ether anesthesia 8 males and 8 females were fixed by intracardiac perfusion with a mixture of allyl alcohol-glutaraldehide (glutaraldehide 2.5%, allyl alcohol 1M in cacodylate buffer) as described by McRae-Degueurce and Geffard (1986). Pituitaries were postfixed in the same solution for three days. After embedding in paraffin wax the pituitaries were cut in the sagital plane and mounted on glass slides.

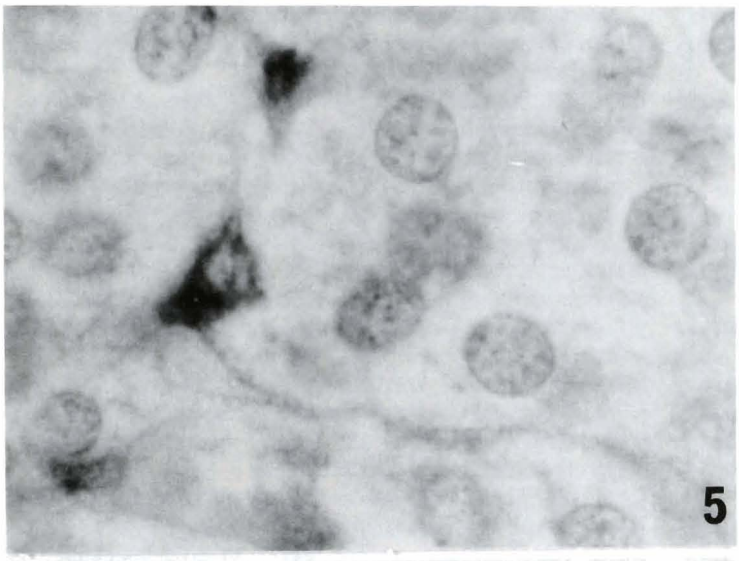
Immunohistochemical staining was performed using the peroxidase-antiperoxidase (PAP) method (Sternberger et al., 1970). Sections were allowed to react with: 1) antibody to 5-HT (1:1500, at 4° C over night) (Chemicon International Inc.); 2) anti-rabbit IgG (1:100, 60 min) (Dako); and 3) PAP (1:100, 30 min) (Dako). The final reaction product was visualized with 3-3'-diaminobenzidine tetrahydrochloride.

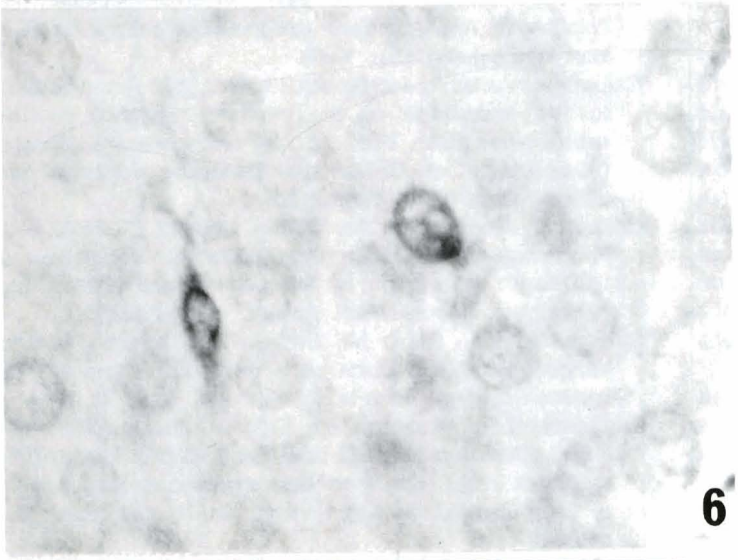
Samples were studied and photographed with a Leitz-Dialux EB 20 light microscope.

Offprint requests to: Dr. J. C. Carvajal, Departamento de Anatomía, Colegio Universitario de Medicina, C./ Santo Tomás s/n, 05003 Avila, Spain



Figs. 1-4. 5-HT labelled fibres in the rat IL. Serotoninergic fibres (arrows) can be seen detaching from the interlobular spaces in a radiated pattern (Fig. 1). These fibres form complex arrangements among the IL cells (Fig. 2). Strongly stained nerve endings can be seen making neuroglandular contacts (arrowheads) (Figs. 3-4). (Figs. 1 and 2, × 5,500; Figs. 3 and 4, × 6,500)





Figs. 5-7. Irregularly shaped 5-HT labelled perikarya can be found in the IL of the rat pituitary, some of them showing distinct cytoplasmic processes (short arrows) (Fig. 7). (Figs. 5 and 7, × 6,500; Fig. 6. × 5,500

Results

With the use of the indirect immunoperoxidase technique in paraffin-embedded sections, we observed both nerve fibres and cell bodies stained with 5-HT antiserum in the intermediate lobe of the rat pituitary. Immunoreaction product was also present in the anterior and posterior lobes of the pituitary as well as in the marginal layer of the pituitary cleft. No sex difference was found.

Nerve fibres

5-HT labelled fibres were realitively scarce in the IL and generally, thin and lightly stained. These labelled fibres appeared to be entering the IL from the posterior lobe, through the interlobular spaces, branching out afterwards in a somewhat radiated pattern (Fig. 1). Complex arrangements of these fibres could be seen among the IL cells (Fig. 2). Fibre endings had stronger immunostaining and showed irregular varicosities. Many of these varicosities were found in close contact with the cell surface making neuroglandular contacts (Figs. 3, 4).

Cell bodies

Cells reactive to anti-5-HT sera were usually heavily stained. These cells were irregularly shaped; polygonal (Fig. 5), oval and fusiform cells (Fig. 6) could be seen. Some of the 5-HT-labelled cells had distinct cytoplasmic processes that sometimes diverge from the cell body between the surrounding cells (Fig. 7).

Discussion

The intermediate lobe has a limited vascular supply (Murakami et al., 1985; Carbajo-Pérez et al., 1989). Consequently, functional control of the IL must be conveyed through neural projections from the central nervous system. It is only recently that significant advances have been made in the knowledge of neuronal projections to the IL, since the first descriptions by Cajal (1894). Stutinsky et al. (1973) have described peptidergic projections from the NL entering the IL. These fibres make contact with glandular cells in the IL. Both DA and GABA have an important role in regulating IL function (Briaud et al., 1979; Demeneix et al., 1984; Taleb et al., 1986) Vuillez et al. (1987) have reported the coexistence of DA and GABA within the same fibres in the IL of

rats. These fibres, which are widely distributed throughout the IL, make synaptoid contacts with the glandular cells (Carbajo et al., 1989).

Westlund and Childs (1982) and Friedman et al. (1983) showed a relatively dense plexus of nerve fibres and terminals stained with 5-HT antiserum in the IL of the rat. Our results, as well as those of Payette et al. (1985) show, on the contrary, that 5-HT labelled fibres are rather scarce in the IL. Differences in the total number of 5-HT-labelled fibres might be due to differences in the technical procedure.

Whether or not 5-HT labelled fibres contain any other neurotransmitter has not yet been confirmed. It is tempting to suggest that 5-HT fibres do not contain other neurotransmitters as the distribution of these fibres greatly differs from that of the catecholaminergic (Payette et al., 1985) and GABAergic fibres (Carbajo et al., 1989). However, the coexistence of glutamate decarboxilase (GAD) and 5-HT in the same perikaryon has been demonstrated in the raphe nuclei by Belin et al. (1983).

We have found cells of a varied morphology reactive to 5-HT serum in the IL of the rat. Some of these 5-HT labelled cells have stellate shape and conspicuous cytoplasmic processes, resembling follicle stellate (FS) cells as described by Amat et al. (1976). Moreover, we have found 5-HT immunoreactivity in the marginal layer of the pituitary cleft. Cells of the marginal layer and FS cells have similar morphological and immunohistochemical features and can be considered as the same cell type (Shirasawa et al., 1983). Therefore, some of the 5-HT labelled cells might be regarded as FS cells.

Serotoninergic perikaryon have been described in the anterior lobe of the pituitary in lower vertebrates (Kondo et al., 1983; Kah and Chambolle, 1983). In mammals, 5-HT has been found in small granules within the pituitary gonadotropes of mice and hibernating bats (Payette et al., 1985). Hence, it cannot be ruled out that some of the cells containing 5-HT in the rat IL are ectopic cells from the anterior lobe, which is not an infrequent finding particularly in gonadotropes.

It is not yet clear if 5-HT labelled cells produce and release 5-HT themselves or, on the contrary, they take in 5-HT from nerve terminals entering the IL from the central nervous system. Both possibilities are likely to be true. Further studies are needed to confirm either of the two hypothesis and their functional meaning.

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