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Original Articles

Immunocytochemical Localization of a Novel Pituitary Protein (7B2) within the Rat Brain and Hypophysis[1]

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A novel pituitary protein called 7B2 was localized in rat pituitary and brain by immunocytochemistry (unlabeled antibody technique). Immunoreactive material was present in the secretory cells of anterior and intermediate lobes and in neural structures of the posterior lobe of the hypophysis. 7B2-immunoreactive neurons were evident within the hypothalamus in the supraoptic nucleus, paraventricular nucleus (magnocellular and parvocellular parts), and lateral hypothalamus. Immunoreactive nerve fibers were seen within the internal and external zone of the median eminence. Among extrahypothalamic regions, the substantia nigra, dorsal tegmental nucleus, cuneiform nucleus, dorsal parabrachial nucleus, spinal tract trigeminal nerve, interior olive, solitary nucleus, and layers I and II of the spinal cord contained 7B2-immunoreactive material. This anatomical distribution suggests a role for 7B2 in endocrine and autonomic functions.

KEY WORDS: Immunocytochemistry; Immunopreadsorption; Novel pituitary protein; Anatomical organization; Hypophysis; Brain.

Introduction

Systematic biochemical analysis of extracts from human and porcine hypophysis has led to the isolation and characterization of a novel pituitary protein (designated 7B2), belonging to a new superfamily (5,16).

The 7B2 identified in extracts from human and porcine pituitaries elutes at similar organic solvent compositions on high-performance liquid chromatography (HPLC), and shows similar isoelectric points (pI 4.9) and molecular weights (21,000) as verified by sodium dodecyl sulfate–polyacrylamide electrophoresis (SDS–PAGE). Moreover, the amino acid composition and sequence of the *N*-terminal 77 and 81 amino acids (human and porcine homologs respectively) revealed a high degree of sequence conservation (16).

Immunocytochemical studies using antibodies generated against a synthetic fragment of this protein show the presence of immunoreactive material within the anterior and posterior lobes of the pituitary and within the neuronal soma of the supraoptic nucleus of the hypothalamus (16).

Radioimmunoassay (RIA) studies confirmed the presence of immunoreactive 7B2 (7B2-IR) in the hypothalamus and pituitary, and also indicated its presence in other regions of the brain as well as in the thyroid and adrenal glands (6,7).

We now report in detail the localization of 7B2-IR within the brain and spinal cord.

Material and Methods

Animals. Male and female Sprague-Dawley rats 8 and 22 days old and adult were used without any prior treatment or after colchicine administration (100 μg/10 μl in distilled water into the lateral ventricle 48 hr before sacrifice). The coordinates were L—1.4 mm, H—7.0 mm, A—7.3 mm, from the stereotaxic atlas of the rat brain (1). The animals were anesthetized with Somnotol prior to injection or at sacrifice.

Fixation and tissue processing. Cold Bouin's solution was chosen from among the fixatives assessed, and administered by cardiac perfusion after washing with ice-cold 0.9% NaCl. The whole brain including the medulla oblongata and pituitary was removed, minced, preserved in fixative for 12 hours at 4°C, dehydrated via a series of alcohols followed by xylene, embedded in paraffin, cut in 5-μm sections, and mounted onto the microscopy slides. The sections from different pituitaries were mounted together on the same slide.

Antiserum and controls. The antiserum against the synthetic 23–39 fragment of 7B2 was raised in rabbits. The sequence of

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$7B2_{23-39}$ is as follows: Glu-Gln-Leu-Gly-Ile-Ala-Arg-Pro-Arg-Val-Glu-Tyr-Pro-Ala-His-Gln-Ala. The peptide was coupled to bovine thyroglobulin at a molar ratio of 10:1 using a water-soluble carbodiimide method (16). Following three boosts, the titer in one of the animals was found to be 1:10,000. This antibody was found to cross-react (20%) with the porcine and human 7B2, but did not recognize any POMC segment including *N*-terminal, adrenocorticotropic hormone (ACTH), α-melanocyte stimulating hormone (α-MSH), β-LPH, β-endorphin (7,16), *o*-corticotropin releasing factor (*o*CRF), 8-arginine vasopressin (AVP), or oxytocin (7).

For immunocytochemistry, immunopreadsorption was performed with synthetic 7B2 both in liquid and solid phase; 1 μg of synthetic antigen in 100 μl of antiserum diluted 1:1000 resulted in total inhibition of immunostaining.

The specificity of antibodies was verified by observation of possible cross-reactivity using solid-phase immunopreadsorption (22) using the following peptides bound to agarose beads: oxytocin, 8-arg-vasopressin, 8-lys-vasopressin, neurophysin, substance P, met-enkephalin, and somatostatin 14; all peptides were used at a concentration of 2 μg/ml in antiserum diluted 1:1000. The same test was performed using thyroglobulin in the range of 0.01–10 μg/ml, and the results were compared with those obtained after immunopreadsorption using synthetic 7B2. Ethanolamine was used to obtain a standard background. The beads were incubated with 4 ml of antiserum to 7B2 diluted 1:1000 for 16 hours at 4°C with shaking. Antisera were then aspirated and used for incubation with tissue sections. The beads, washed three times for 10 min with phosphate-buffered saline (PBS), were exposed to fluorescein isothiocyanate (FITC) in a dilution of 1:30 for 45 min at 37°C, washed again with PBS three times for 10 min, resuspended in 1 drop of 90% glycerol/PBS, and deposited on a glass slide, which was examined by Leitz (UV equipped) microscopy. Photographs were taken with the same exposure time for all samples (25 sec).

Immunostaining. The unlabeled antibody technique of Sternberger (20) was used. The rehydrated sections were successively exposed to normal goat serum (NGS) 3% in PBS for 15 min and to nonpreadsorbed and preadsorbed antisera diluted 1:1000 in PBS containing 1% NGS for 24–48 hours at 4°C. After three washes for 10 min with PBS, the antirabbit IgG, diluted 1:60 in 10% NGS/PBS, was applied for 30 min, followed by three washes for 10 min with PBS. The sections were exposed to the peroxidase–antiperoxidase (PAP) complex diluted 1:60 in 1% NGS for 30 min, then washed and allowed to react with diaminobenzidine (30 mg/100 ml Tris-HCl buffer + 100 μl of 30% H_2O_2) for 10 min. As a control, the antiserum was omitted and nonimmune rabbit serum was applied.

Results

Specificity of Antiserum

The antiserum incubated with different heterologous peptides bound to agarose beads induced a faint fluorescence comparable to that obtained with ethanolamine as a background (Figure 1a–h). Immunostaining was not affected by incubation of sections with these preadsorbed antisera. This observation allowed us to conclude that there was no cross-reaction between 7B2 antiserum and oxytocin, lys-vasopressin, arg-vasopressin, neurophysin, somatostatin, substance P, or met-enkephalin. The thyroglobulin bound to agarose appeared to be more potent in attracting some of the antibodies, since the intensity of generated fluorescence increased in a range of concentrations running from 0.1 to 10 mg/1 ml (Figure 1i and j), but never attained the intensity observed with 7B2 (Figure 1k). The use of the thyroglobulin immunopreadsorbed antiserum resulted in partial decrease of staining and background, but did not affect the visualized structures. The synthetic 7B2 complexed with agarose induced a strong fluorescence (Figure 1k). The immunopreadsorbed antiserum did not show the staining (Figure 2b). No reaction was found when the antiserum was omitted or when preimmune rabbit serum was used.

Distribution of 7B2-IR in Pituitary

In all rat groups (8 and 22 days old and adult), an immunoreaction was observed in the anterior (AL), intermediate (IL), and posterior (PL) lobes (Figure 2a,c,d). Immunoreactive cells were sparsely distributed in the AL, attached to sinusoids and within strongly stained cells lying on the border of the lobe (Figure 2c).

All cells of the intermediate lobe were labeled. Sometimes the dorsal part of the lobe exhibited more intense PAP staining (Figure 2d). The best staining was obtained after 48 hr of incubation.

Immunostaining was localized in the posterior pituitary, associated with fibers surrounding the vessels and in adult rats within the nerve swellings, which were densely packed with reaction product (Figure 2d).

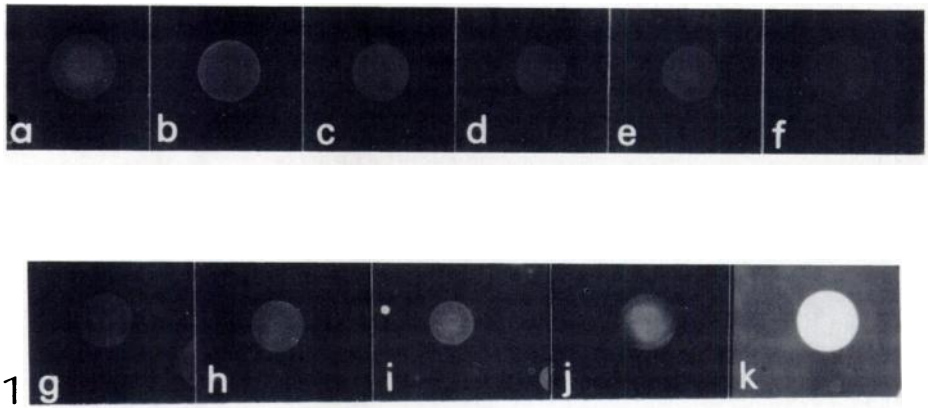


Figure 1. Solid-phase immunopreadsorption of 7B2 antiserum diluted 1:1000 using ethanolamine as a standard (**a**) and the following peptides: oxytocin (**b**), arg-vasopressin (**c**), lys-vasopressin (**d**), neurophysin (**e**), substance P (**f**), met-enkephalin (**g**), and somatostatin (**h**). No fluorescence was observed. Thyroglobulin bound to the agarose beads generated fluorescence, increasing over the range 0.1–10 μg/1 ml of diluted antiserum (**i** and **j**). Adsorption with synthetic 7B2 produced a strong fluorescence (**k**) under the same conditions.

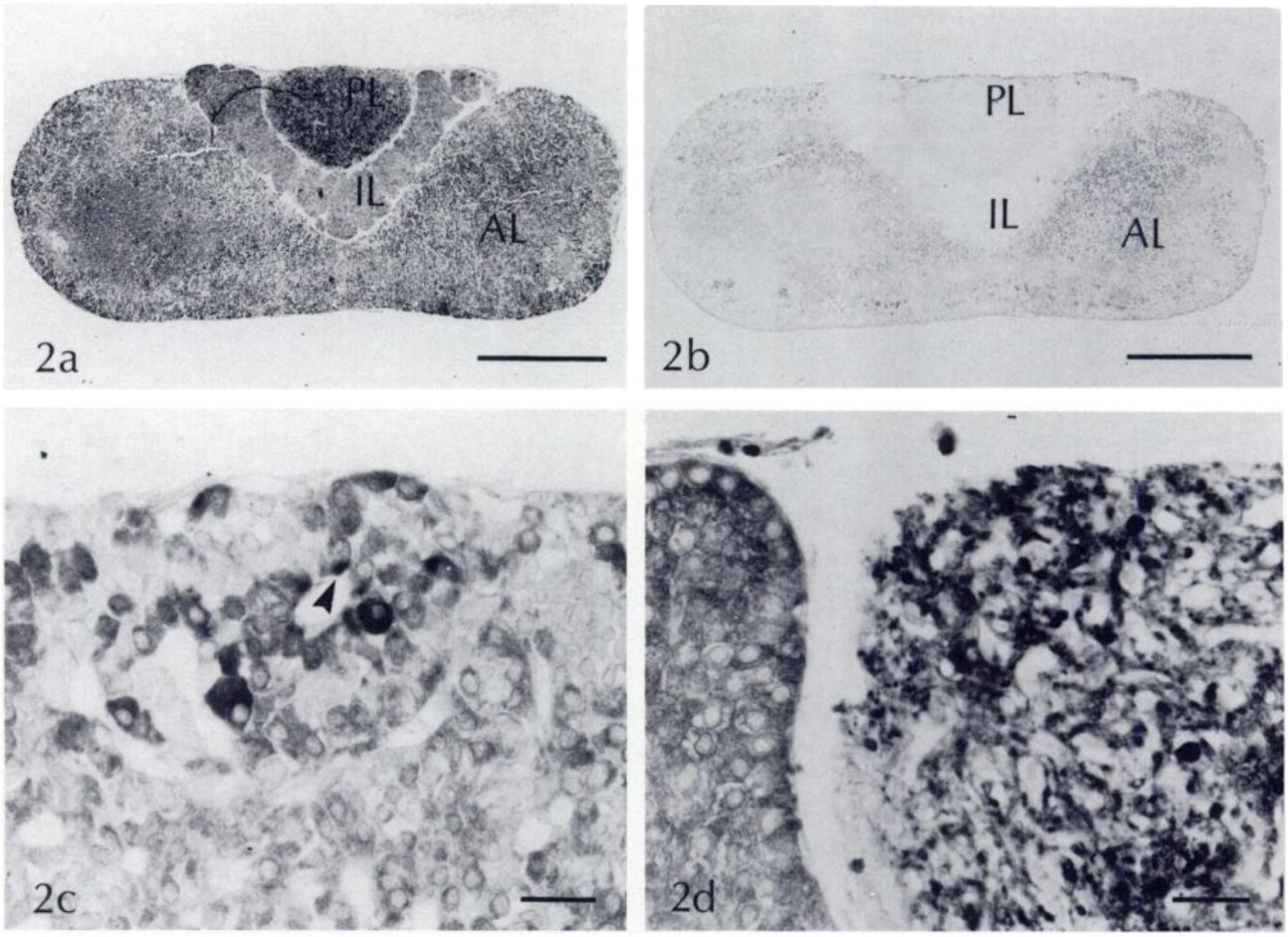


Figure 2. (a,b) Adult rat pituitary gland: paraffin-embedded specimen, 5-μm successive sections. Low-power micrographs of immunostaining obtained after 48 hr of incubation with 7B2 antiserum 1:1000. In a, the sites of reaction are largely distributed in the whole organ; b shows the effect of immunopreadsorption of antiserum with synthetic 7B2 fragment. The reaction is completely abolished within the whole organ, except for some nonspecific background in the anterior lobe. AL = anterior lobe, IL = intermediate lobe, PL = posterior lobe. Original magnification ×17. Bar = 1000 μm. (c) Adult rat: higher magnification of adenohypophysis. The immunoreaction product is found within the cells as dispersed or granular material surrounding the nuclei. Note the apical accumulation of PAP deposit in cells associated with the sinusoid. Original magnification ×400. Bar = 25 μm. (d) Adult rat: higher magnification of posterior lobe (right) showing the sections of nerve fibers and terminal swellings labeled with PAP reaction product. The intermediate lobe cells (left) exhibit a rather moderate immunoreaction within the cytoplasm. Original magnification ×400. Bar = 25 μm.

The intensity of reaction varied with age. Among the pituitaries studied, those from animals of 8 and 22 days exhibited a more intense staining of the AL cells than in the adult. In contrast, the intensity of staining within the PL seems to increase from moderate in neonatal and young rats to strong in the adult rat (Table 1), whereas that in the intermediate lobe does not seem to change with age.

Distribution of 7B2-IR in Hypothalamus

In all groups of untreated animals, the immunoreactive material was found in the neurons of the supraoptic nucleus (SON) (Figure 3a and b), within the fibers of the median eminence (ME) (Figure 4), and in the hypophyseal stalk. Excluding the above regions, the PAP deposit was not observed in neonatal and young rats, whereas in adults the immunoreaction was found to be widely distributed. Granules of different forms and sizes were seen in several areas of the hypothalamus: the paraventricular nucleus, both magnocellular (PVOm) and parvocellular (PVOp), the lateral hypothalamic area (LHy), and the area perifornicalis (AP) (Table 2).

Administration of colchicine resulted in the appearance of strongly immunostained neuronal somas inside structures adjacent to the ventricle. Colchicine treatment increased the immunoreaction within the cells of the supraoptic nucleus (Figure 3b). Sparsely distributed positive neurons were found in the entire rostral hypothalamus. In particular, neurons associated with capillaries were frequently seen in the lateral hypothalamus (Figure 3a), and supraoptic retrochiasmic area (SONr) (not shown). The paraventricular nuclei exhibited 7B2-

Table 1. *Intensity of 7B2-IR labeling observed in pituitary of neonatal, young, and adult rats*[a]

Age of rat	Pituitary lobe		
	Anterior	Intermediate	Posterior
8 days	+ + + + +	+ +	+ +
22 days	+ + + + +	+ +	+ +
Adult	+ + +	+ +	+ + + + +

[a]Intensity of labeling increases from + to + + + + +. Results were obtained from four rats in each group. Sections representing all ages were mounted together and scored comparatively.

IR within the magnocellular neurons, and parvocellular neurons were also stained (Figure 3a and c).

Extrahypothalamic Distribution of 7B2-IR

The concentration of immunostained material was lower outside the hypothalamus. The regions that exhibited a positive reaction in adult rats were the medial pars of the substantia nigra, pars reticulata and compacta (SN) (Figure 5a). There the reaction product formed irregular granules, often arranged with faintly visible fibers in a reticular pattern (Figure 5b). In the midbrain, the whole periaqueductal griseum contained a weak, finely dispersed reaction product. The strongest concentration of immunoreaction was evident within the dorsal tegmental nucleus (DTN), cuneiform nucleus (CN) and dorsal parabrachialis nucleus (DPN) (Figure 6). The staining of all these structures contrasted with that of the white matter, areas of the cortex, and inferior colliculus, which were unstained. At higher magnification, more or less dense irregular granules were seen distributed through the parenchyma (not shown).

Among the pontine structures, the spinal tract trigeminal nerve (STTN), inferior olive (IO), and nucleus solitarius (NS) were strongly labeled (Figure 7a). Figure 7b shows an example of IO staining to illustrate the typical aspect of the varicosity-like and/or terminal-like structures crossing the parenchyma. Such an aspect was similar to that of the cornu dorsale (CD) of the spinal cord (Figure 8). The IR-7B2 was mainly concentrated in layers I and II, and little immunoreactivity was found in deeper layers.

Discussion

This study shows the immunocytochemical localization of a recently isolated and characterized pituitary protein, 7B2 (16), in the pituitary gland and CNS. The results were obtained using the antiserum previously shown by RIA not to cross-react with adenohypophysis POMC peptides, oxytocin, or vasopressin (7,16). Although the larger 7B2 sequence shows partial homology to proinsulin, Rous sarcoma virus transforming protein, and pig secretin (5), the 23–39 7B2 fragment used to raise the antiserum does not present homology to any of these proteins, including secretin. Secretin immunoreactivity is known to occur in brain and in pituitary gland; however, its cerebral distribution (12) does not correlate with that of 7B2-

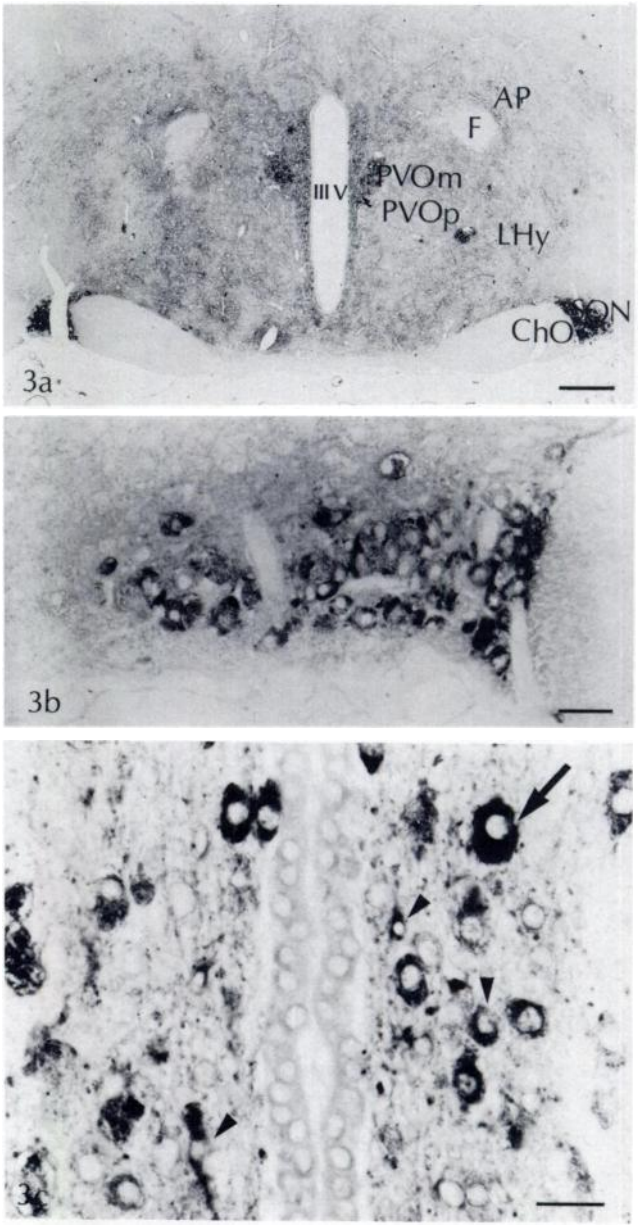


Figure 3. (a) Adult rat treated with colchicine. Low-magnification frontal section through rostral hypothalamus. The PAP deposit widely distributed through the parenchyma contrasts with the negative reaction of white matter. Note the strong reaction within the supraoptic nucleus (SON), the paraventricular nuclei both magnocellular (PVOm) and parvocellular (PVOp), and the group of neurons associated with capillaries in the lateral hypothalamus (LHy). AP = area perifornicalis, ChO = optic chiasm, F = fornix, III V = third ventricle. Original magnification ×20. Bar = 400 μm. (b) Higher magnification view of SON magnocellular neurons. Original magnification ×187. Bar = 40 μm. (c) Detail showing the population of neurons within the PVNp. Most of the neurons are parvocellular (arrowhead); only a few magnocellular neurons are seen (arrow). Original magnification ×235. Bar = 40 μm.

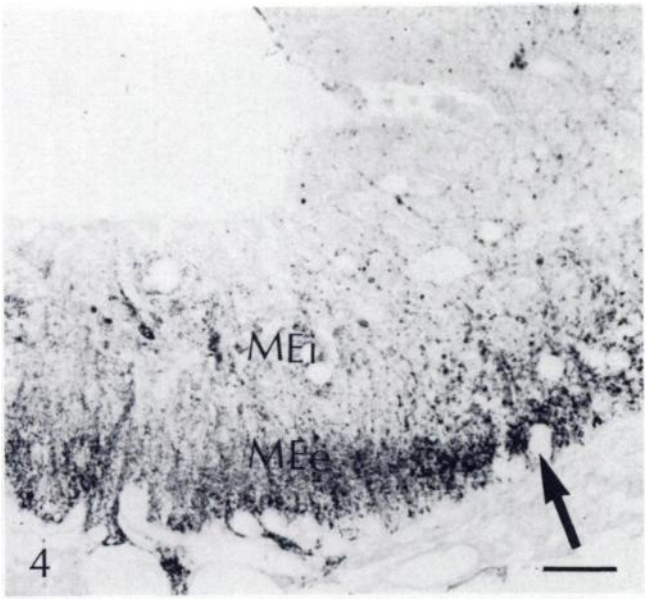


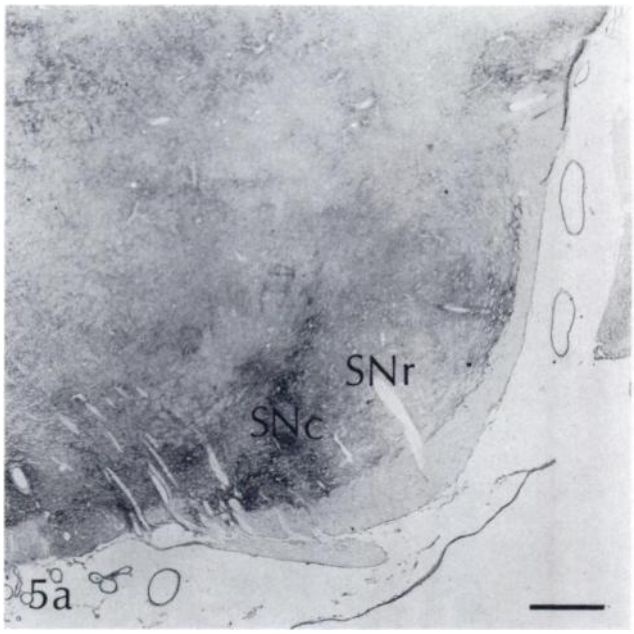
Figure 4. 7B2-IR fibers within the median eminence. Both internal (MEi) and external (MEe) laminae exhibit immunoreaction. Note that the strongest accumulation of 7B2-IR is around portal capillaries and around the perivascular spaces (arrow). Original magnification × 392. Bar = 25 μm.

IR. Since the brain contains other peptides known to have in some particular areas a similar localization to 7B2-IR (23), immunopreadsorption tests were performed using double-control observation on agarose beads treated for immunofluorescence, and simultaneously on tissue sections. Under these conditions no cross reaction was detected with oxytocin, lys-vasopressin, arg-vasopressin, neurophysin, substance P, or met-enkephalin. The minor cross-reactivity with thyroglobulin had no significant effect on specific immunostaining, since preadsorption of antiserum resulted mainly in reducing the background. Moreover, the liquid-phase and solid-phase immunopreadsorption with synthetic 7B2 fragment totally blocked the effect of immunocytostaining and induced strong immunofluorescence of material bound to agarose beads. Therefore, according to generally accepted criteria, the specificity of the 7B2 antiserum (21) was such that all antibodies recognized the antigen and were able to detect antigenic sites in tissue. The three lobes of the pituitary gland contained 7B2-IR material. It was found in the anterior lobe in the cells characterized as gonadotrophs (Capella, Buffa, and Magnoni, and Polak and Bloom, personal communications), and in the posterior lobe, where strongly immunoreactive sites correspond to nerve fibers and endings. In contrast, uniform and moderate immunocytostaining was seen within the intermediate lobe. Since the reaction was displaceable by synthetic 7B2 (but not by thyroglobulin), this confirmed that the antigen is present in all these areas, albeit in different local concentrations. Several factors have led us to report now the immunostaining in the intermediate lobe: 1) the antiserum actually used exhibits less nondisplaceable background, 2) the maximum immunostaining intensity was obtained not earlier than after 48 hr of incubation, 3) use of Sternberger's technique has been shown to be more sensitive than the indirect immunoperoxidase procedure. These conditions most clearly permitted us to appreciate the effect of staining and its total displacement. The neurohypophysis 7B2-IR originates from hypothalamic nuclei.

Table 2. *Distribution of 7B2-IR material in CNS in normal and colchicine-treated rats*[a].

Structure	Normal Rats			Colchicine-treated rats
	8 days	22 days	Adult	Adult
Hypothalamic distribution				
Supraoptic nucleus	W	W	W	S
Supraoptic nucleus, retrochiasmic area	—	—	G	S
Paraventricular nucleus, magnocellular	—	—	G	S
Paraventricular nucleus, parvocellular	—	—	G	S
Lateral hypothalamus	—	—	G	S
Area perifornicalis	—	—	G	G
Median eminence	G	G	G	G
Hypophyseal stalk	G	G	G	G
Extrahypothalamic distribution				
Substantia nigra, ventral part	—	—	G	G
Central gray matter	—	—	G	G
Dorsal tegmental nucleus	—	—	G	G
Cuneiformis nucleus	—	—	G	G
Dorsal parabrachialis nucleus	—	—	G	G
Spinal tract trigeminal nerve	—	—	G	G
Inferior olive	—	—	G	G
Nucleus tractus solitarius	—	—	G	G
Cornu dorsale, laminae I–II	—	—	G	G

[a]—No reaction; G = granular deposit of PAP product in cerebral parenchyma. PAP deposit within the neurons: W, weak and S, strong intensity of reaction.



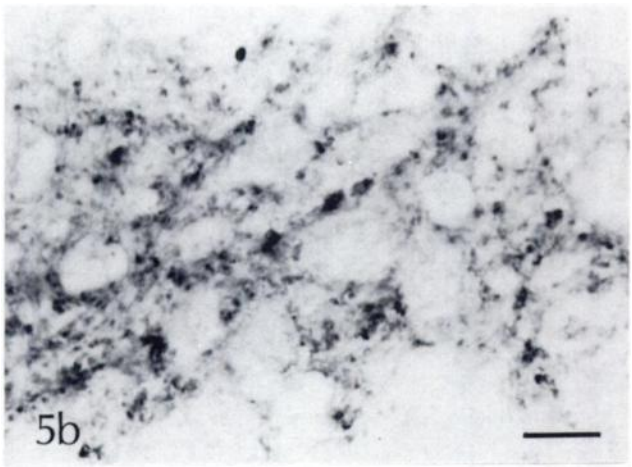


Figure 5. (a) Low magnification frontal section through mesencephalon. 7B2-IR labels the medial pars of the substantia nigra (SNc and SNr). Original magnification × 24.5. Bar = 400 μm. (b) Higher magnification view showing the PAP deposit in the SNc. The small and irregular granules are arranged in the form of a varicosity-like reticulum. Original magnification × 392. Bar = 25 μm.

It is not yet evident whether in the anterior and intermediate lobes immunoreactive material originates from local synthesis or uptake. Although little is yet known about the origin, synthesis, and function of 7B2 protein within the pituitary, the protein seems to be linked to its secretory activities, since the anatomic distribution selectively covers the sites of glandular and neurosecretory structures. The ontogeny of 7B2 in the AL and PL seems to be different, since the 7B2-IR staining in the anterior hypophysis is more intense in neonatal and young rats than in the adult, whereas the immunoreactivity of

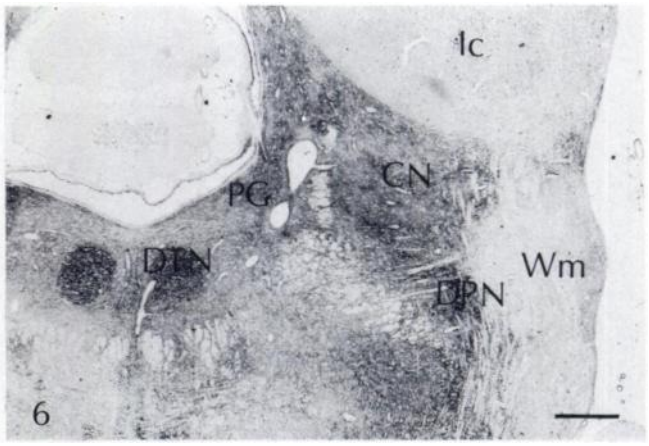
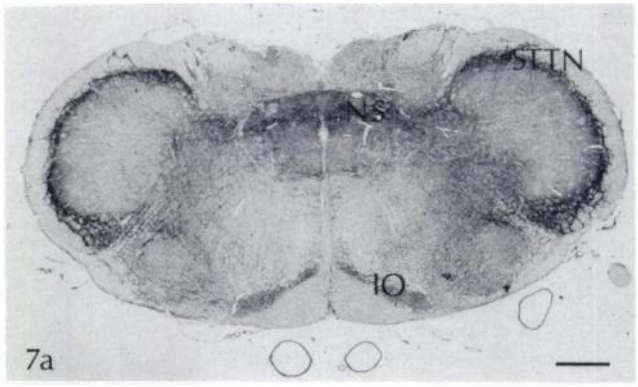
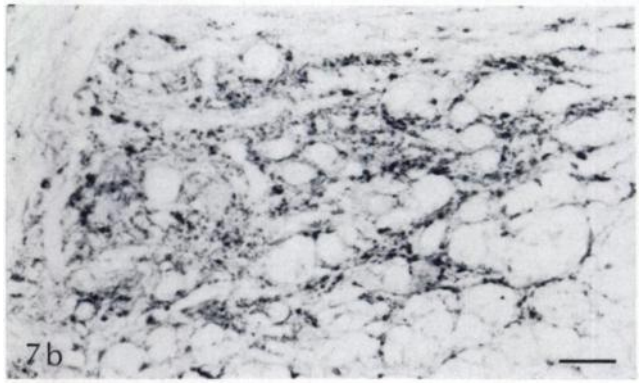


Figure 6. Macrophotograph through the midbrain frontal section. PAP deposit is seen in the whole periaqueductal griseum (PG). The strongest labeling is observed within symmetrically located structures: the dorsal tegmental nucleus (DTN), cuneiform nucleus (CN), and the region of the dorsal parabrachialis nucleus (DPN). Labeling was absent in the mass of white matter (Wm) and inferior colliculus (Ic). Original magnification × 9. Bar = 1000 μm.

Figure 7. (a) Macrophotograph of pontine region. Spinal tract trigeminal nerve (STTN), inferior olive (IO) and nucleus solitarius (NS) are labeled with 7B2 antiserum. Original magnification × 7.5. Bar = 1000 μm. (b). Higher magnification of the fragment of the IO region illustrates the existence of varicosity-like structures. Original magnification × 304. Bar = 25 μm.





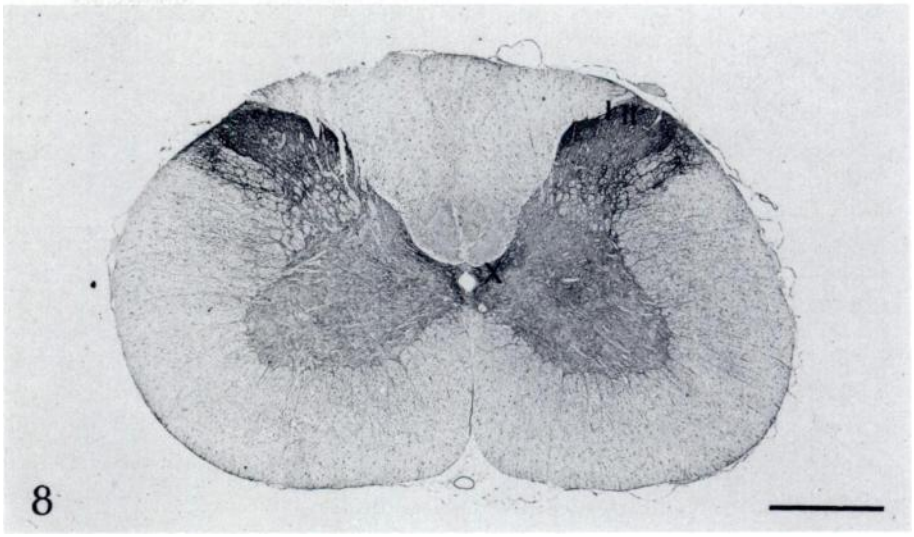


Figure 8. Macrophotograph of section from cervical part of spinal cord. Weak 7B2-IR labeling covers the whole area of the gray matter. The strong reaction is localized within layers I and II of the dorsal horn containing the primary sensory neurons. In deeper layers only laminae X show moderate labelling. Original magnification × 15. Bar = 1000 μm.

neural hypophysis increases with age. Moreover, there is a good correlation between the increase of 7B2-IR in the whole brain and in the neurohypophysis because the only sites clearly visualized in newborn and young rats were the SON and ME. Other cerebral regions containing 7B2-IR have been observed only at maturity.

These regional and quantitative differences could indicate the differential participation of this protein in glandular and neural activity. Several peptides are known to exert a different role in the hypophysis and in nervous tissue. Good examples are oxytocin and vasopressin, hormones that can alter the electrical activity in the CNS (4,10,11).

Without any pretreatment, the brown granular PAP deposit (concentrated or diffused) showed the presence of the 7B2 antigen in different cerebral regions. However, the cellular localization made possible by colchicine administration resulted in the appearance of intense staining, mostly in magnocellular neurons within the SON, SONr, PVNm, LHy, and also in parvocellular neurons in the PVNp.

The interpretation of PAP-positive results for regions distal to the ventricle (i.e., the PA) and for the extrahypothalamic areas (which were not affected by colchicine) is still uncertain with regard to the 7B2-IR localization within the perikarya or within the terminals. Nevertheless, the effect of colchicine indicates that 7B2-IR protein may be synthesized within the neuronal somas of hypothalamic nuclei and transported via axonal flow flux to their respective targets. The projections coming from the SON are almost exclusively to the PL (13), but those of the PVO were clearly demonstrated to connect in addition with the hindbrain and spinal cord areas (8,14). Our observations, for which light microscopy does not provide adequate resolution, will be corroborated by future study, analogous to that of Conrath-Verrier et al. (3), with local colchicine administration to the discrete CNS nuclei. Under these conditions and using electron microscopy it should be possible to elucidate whether 7B2-IR is present within the perikarya or within the terminals contacting the target neurons in the regions described above (PA, SN, PG, DTN, CN, STTN, IO, NS, CD).

The positively stained neurons are distributed throughout the hypothalamic NSO and PVO nuclei. Both nuclei are known to be related via an internal zone of the ME to the neurohypophysis (15). Furthermore, the PVO projections contact the portal capillaries of the ME external zone (2,9,28). This system is involved in the regulation of adenohypophysial activity. Certain pathways of the PVO contact with autonomic centers of the brain system and spinal cord and, there, are involved in a range of aspects of central autonomic regulation, nociception, and behavior (18,19).

The correlation of this fact with the anatomic distribution of 7B2-IR gives more support to the hypothesis that 7B2 protein could participate in endocrine and autonomic functions.

Immunoreactivity to several peptides has been demonstrated in the magnocellular division of both the NSO and the PVN, namely oxytocin, vasopressin, and their associated neurophysins, met-enkephalins and leu-enkephalins, gastrin, dynorphins, renin, and glucagon (see extensive review by Swanson and Sawchenko, ref 23). Among these peptides, the dynorphins, the differential proteolytic processing products of prodynorphin B, have been reported within anterior and neurointermediate pituitary lobes in various concentrations (17).

Moreover, the immunoreactivity of the prodynorphin-B-derived peptides exhibited a common localization with vasopressin in neurons and projections in the brain (24,25,26,27). Furthermore, preliminary data in our laboratory show the common localization of 7B2, arg-vasopressin, and dynorphin 1–13 immunoreactivities within the SON, PVO, and posterior pituitary. Interestingly, the whole area of 7B2-IR is larger and extends to regions where arg-vasopressin is absent (the intermediate and anterior pituitary lobes). Thus, we suggest that

7B2-IR may be in part associated with dynorphin and/or vasopressin systems; however, this association appears to be irregular. We expect that further investigation will yield an analysis of the above question.

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Literature Cited

1. Albe-Fessard D, Stutinsky F, Libouban S: Atlas Stéréotaxique du Diencéphale du Rat Blanc. 2nd Ed. CNRS, Paris, 1971
2. Antunes JL, Carmel PW, Zimmerman EA: Projections from the paraventricular nucleus to the zona externa of the median eminence of the rhesus monkey: An immunohistochemical study. Brain Res 137:1, 1977
3. Conrath-Verrier M, Dietl M, Arluison M, Cesselin F, Bourgoin S, Hamon M: Localization of met-enkephalin-like immunoreactivity within pain-related nuclei of cervical spinal cord. Brain Res Bull 11:587, 1983
4. Gilbey MP, Coote JH, Fleetwood-Walker S, Peterson DF: The influence of the paraventriculo-spinal pathway and oxytocin and vasopressin on sympathetic preganglionic neurones. Brain Res 241:43, 1982
5. Hsi KL, Seidah NG, De Serres G, Chrétien M: Isolation and NH_2-terminal sequence of a novel porcine anterior pituitary polypeptide. Homology to proinsulin, secretin and Rous sarcoma virus transforming protein TVFV60. FEBS Lett 147:261, 1982
6. Iguchi H, Chan JSD, Dennis M, Seidah NG, Chrétien M: Regional distribution of a novel pituitary protein (7B2) in the rat brain. Brain Res, in press
7. Iguchi H, Chan JSD, Seidah NG, Chrétien M: Tissue distribution and molecular forms of a novel pituitary protein in the rat. Neuroendocrinology 39:453, 1984
8. Kuypers HGJM, Maiskey VA: Retrograde axonal transport of horseradish peroxidase from spinal cord to brain stem cell groups in the cat. Neurosci Lett 1:9, 1975
9. Lechan RM, Nestler JL, Jacobson S, Reichling S: The hypothalamic tuberoinfundibular system of the rat as demonstrated by horseradish peroxidase (HRP) microiontophoresis. Brain Res 195:13, 1980
10. Morris R, Salt TE, Sofroniew MV, Hill RG: Actions of microiontophoretically applied oxytocin, and immunohistochemical localization of oxytocin, vasopressin and neurophysin in the rat caudal medulla. Neurosci Lett 18:163, 1980
11. Mühlethaler M, Dreifuss JJ, Gähwiler BH: Vasopressin excites hippocampal neurones. Nature (London) 296:749, 1982
12. O'Donohue ThL, Charlton CG, Miller RL, Boden G, Jacobowitz DM: Identification, characterization, and distribution of secretin immunoreactivity in rat and pig brain. Proc Natl Acad USA 78:5221, 1981
13. Rasmussen AT: Effect of hypophysectomy and hypophysial stalk resection on the hypothalamic nuclei of animals and man. Assn Res Nerv Ment Dis 20:245, 1940
14. Saper CB, Loewy AD, Swanson LW, Cowan WM: Direct hypothalamo-autonomic connections. Brain Res 117:305, 1976
15. Saper CB, Swanson LW, Cowan WM: The efferent connections of the anterior hypothalamic area of the rat, cat and monkey. J Comp Neurol 182:575, 1978
16. Seidah NG, Hsi KL, De Serres G, Rochemont J, Hamelin J, Antakly T, Cantin M, Chrétien M: Isolation and NH_2-terminal sequence of a highly conserved human and porcine pituitary protein belonging to a new superfamily. Immunocytochemical localization in pars distalis and pars nervosa of the pituitary and in the supraoptic nucleus of the hypothalamus. Arch Biochem Biophys 225:525, 1983
17. Seizinger BR, Grimm C, Höllt V, Herz A: Evidence for a selective processing of pro-enkephalin B into different opioid peptide forms in particular regions of rat brain and pituitary. J Neurochem 42:447, 1984
18. Sofroniew MV: Morphology of vasopressin and oxytocin neurones and their central and vascular projections. In The Neurohypophysis: Structure, Function and Control, Progress in Brain Research. Edited by BA Cross, G Leng. Elsevier, New York, 1983, p 101–114
19. Sofroniew M: Vasopressin and oxytocin in the mammalian brain and spinal cord. Trends Neurosci 6:467, 1983
20. Sternberger LA (ed): Immunocytochemistry. 2nd Ed. Wiley, New York, 1979
21. Swaab DF: Comments on the validity of immunocytochemical method. In Cytochemical Methods in Neuroanatomy, Neurology and Neurobiology, Vol 1. Edited by V Chan-Palay and SL Palay. Alan Liss, New York, 1982, p 423–440
22. Swaab DF, Pool CW: Specificity of oxytocin and vasopressin immunofluorescence. J Endocrinol 66:263, 1975
23. Swanson LW, Sawchenko PE: Hypothalamic integration: Organization of the paraventricular and supraoptic nuclei. Ann Rev Neurosci 6:269, 1983
24. Watson SJ, Akil H, Fischli W, Goldstein A, Zimmerman E, Nilaver G, Van Wimersma Greidanus TB: Dynorphin and vasopressin: Common localization in magnocellular neurons. Science 216:85, 1982
25. Watson SJ, Khachaturian H, Taylor L, Fischli W, Goldstein A, Akil H: Pro-dynorphin peptides are found in the same neurons throughout rat brain: Immunocytochemical study. Proc Natl Acad Sci USA 80:891, 1983
26. Watson SJ, Seidah NG, Chrétien M: The carboxy terminus of the precursor to vasopressin and neurophysin: Immunocytochemistry in rat brain. Science 217:853, 1982
27. Weber E, Barchas J: Immunohistochemical distribution of dynorphin B in rat brain: Relation to dynorphin A and α-neo-endorphin systems. Proc Natl Acad Sci USA 80:1125, 1983
28. Weigand SJ, Price JL: The cells of origin of the afferent fibers to the median eminence in the rat. J Comp Neurol 192:1, 1980