Immunohistochemical Studies on the Peptidergic Nerve Fibers in the Pineal Organ of the Dog

Tadao MATSUURA, Mitsuhiro KAWATA, Hisao YAMADA, Munekado KOJIMA and Yutaka SANO

Department of Anatomy (Prof. Y. SANO), Kyoto Prefectural University of Medicine, Kyoto, Japan

Received October 4, 1982

Summary. The distribution of vasopressin-, oxytocin- and LHRH-containing nerve fibers in the pineal organ of the dog was demonstrated by use of the peroxidase-antiperoxidase immunohistochemical technique. These neuropeptide-containing fibers penetrated through the pineal stalk from the brain, mainly from the posterior commissural region, into the pineal organ. The vasopressin fibers were the most prominent in number, oxytocin fibers and LHRH fibers were the least. Most of these fibers were found in the proximal part of the pineal organ, but some of them were also observed in the distal part. These peptidergic fibers were distributed not only in the perivascular spaces but among the parenchymal cells.

It has been demonstrated by BARGMANN (1954), BARRY (1956), SUOMALAINEN (1960) and OKSCHE (1965), using Gomori's chrome hematoxylin phloxin staining, that the nerve fibers containing neurosecretory material, posterior lobe hormones, occurred in the mammalian pineal organ. Recently BUJIS and PéVET (1980, in the rat) and NÜRNBERGER and KÖRF (1981, in the hedgehog) immunohistochemically demonstrated the distribution of the vasopressin- and oxytocin-containing nerve fibers in the pineal organ. Furthermore, UDDMAN et al. (1980) reported the existence of nerve fibers containing vasoactive intestinal peptide (VIP) in the pineal organ of some mammals, i.e. the rabbit, cat and pig.

Using the retrograde transport method of horseradish peroxidase, KÖRF and WAGNER (1980) suggested the possibility that vasopressin and oxytocin fibers in the pineal organ of the guinea pig form a part of extrahypothalamic neurosecretory system by proving the existence of their perikarya in the paraventricular and suprachiasmatic nuclei.

However, the study on the origin of peptidergic nerve fibers in the pineal organ is in sufficient, and their functional role is still open to discussion.

In the present work, the distribution of peptidergic nerve fibers in the dog pineal organ was studied by using peroxidase-antiperoxidase technique with anti-vasopressin, anti-oxytocin and anti-LHRH antiserum. The functional role of these nerve fibers is discussed.
MATERIALS AND METHODS

Ten adult dogs (mongrel male, 4-5 kg body weight) were used in this study. They were kept under natural light conditions and were killed between 10:00 and 15:00 between March and July. Four animals used in this study were killed 7 to 10 days after removing the bilateral superior cervical ganglia. The dogs were perfused with saline to wash out the blood, followed by perfusion fixation with 4% paraformaldehyde, 0.3% glutaraldehyde and 0.3% picric acid in 0.1 M phosphate buffer at pH 7.4 via the left cardiac ventricle under sodium pentobarbital anesthesia. The tissue blocks including the pineal body, the habenular nucleus, the posterior commissural region and the superior colliculus were dissected out immediately after perfusion fixation. After post-fixation with same fixative mixture without glutaraldehyde for 2 days at 4°C, the tissue blocks were immersed in 20% sucrose in the same buffer for 2 days at 4°C. Parasagittal frozen sections of 20 μm thickness were prepared by cryostat and were stored in 0.1 M phosphate buffered saline (PBS) (pH 7.4) with 0.3% Triton x-100. The solution, PBS with Triton x-100, was used in all staining procedures.

Immunohistochemical staining was performed by the peroxidase antiperoxidase (PAP) method in free floating sections. Sections were immersed in the first antiserum, anti-vasopressin, anti-oxytocin (Immunonuclear Corporation) or anti-LHRH (Otsuka Assay Institute), at a dilution of 1:8,000 for 2 days at 4°C. Then they were immersed in anti-rabbit IgG (Miles-Yeda Ltd.) (1:200) for 2 hrs at room temperature, followed by immersion in PAP (DAKO-immunoglobulins) (1:200) for 2 hrs at room temperature. After reacting with 3,3’ diaminobenzidine, sections were dehydrated, cleared and mounted by Entellan.

RESULTS

Nerve fibers containing vasopressin, oxytocin or LHRH were immunohistochemically demonstrated in the pineal organ of the dog. The pinealocytes showed a negative reaction to these three kinds of antibodies. Vasopressin-, oxytocin- and LHRH-containing varicose fibers were also observed in the habenular and posterior commissural regions (Fig. 1, 4). In these peptidergic nerve fibers vasopressin fibers showed the most dense distribution in the brain parenchyma, while the other two kinds of fibers were observed scattered. It was a remarkable feature of the vasopressin and oxytocin fibers that many fibers gathered in the small area, especially around blood vessels, of the subcommissural organ (Fig. 1). These peptidergic fibers entered the pineal organ mainly through the pineal stalk from the posterior commissural region.

In the pineal organ, most peptide immunoreactive fibers were distributed in its proximal part and some in its middle part (Fig. 1). Many fibers were closely related to blood vessels, but some of them were distributed among the parenchymal cells. A few PAP positive nerve fibers ran within or closely beneath the pineal capsule (Fig. 3). These fibers branched into the middle and distal part of the pineal parenchyma. Each peptidergic fiber showed the same distribution pattern. The total number of these fibers were not many, as shown in the figures. Among the fibers, the vasopressin immunoreactive ones were the most abundant, followed by the oxytocin fibers; the LHRH fibers were the least.

These fibers were beaded in structure showing distinct varicosities. Two types
Fig. 1. Vasopressin fibers in the pineal organ (PO) and in the posterior commissure (PC). The fibers run mainly along blood vessels (*) and in the perivascular spaces (arrow). Bundles of vasopressin fibers are also observed in the adjacent area of the subcommissural organ (SCO). VIII third ventricle. Parasagittal section through the pineal organ and the stalk. ×180

Fig. 2. Thick and thin types of vasopressin varicose fibers in the proximal central part of the organ. *Blood vessel. ×450

Fig. 3. Vasopressin fibers located within and closely beneath the capsule (Cap). Some fibers extend their branches into the parenchyma. ×540
Fig. 4. LHRH fibers in the posterior commissure (PC). A part of them run in the subependymal vascular layer of the subcommissural organ (SCO). * Blood vessels. ×630

Fig. 5. A beaded LHRH fiber in the central part of the pineal organ. ×270

Fig. 6. Thick and thin types of oxytocin varicose fibers in the central part of the pineal parenchyma. ×620
Immunohistochemical Peptidergic Fibers in Dog Pineal Organ

of fibers, thick and thin types, and those of varicosities; large and small types, could be distinguished. Thick-type fibers were predominant in the pineal stalk and the proximal part of the organ. Generally large type varicosities belonged to thick-type fibers (approximately 0.8 μm in diameter) (Fig. 2, 6). In the vasopressin and oxytocin fibers, large type varicosities were 3.1-4.6 μm (average 4.1 μm) in diameter and in the LHRH fibers they measured 2.3-3.5 μm (average 2.7 μm). On the other hand, thin-type fibers (approximately 0.3 μm or less in diameter) were found mainly in the perivascular region. Small-type varicosities belonged to this type of fibers (Fig. 2, 5, 6). In the vasopressin and oxytocin fibers, the diameter of this type of varicosity was 2.3-2.8 μm (average 2.1 μm) and in LHRH fibers, it was 1.4-1.9 μm (average 1.5 μm) (Fig. 5).

No changes were detected on the distribution of the peptidergic fibers in the epithalamic regions, including the pineal and subcommissural organs after a superior cervical ganglionectomy.

DISCUSSION

Due to recent progress in the immunohistochemical technique, certain kinds of peptidergic nerve fibers have been detected in the pineal organ of some mammalian species (vasopressin and oxytocin in the rat: Buijs and Pêvet, 1980; VIP in the rabbit, cat and pig: Uddman et al., 1980; vasopressin and oxytocin in the hedgehog: Nürnberg and Körf, 1981). The present study demonstrates vasopressin-, oxytocin- and LHRH-containing nerve fibers in the canine pineal organ.

Although the origin of the peptidergic fibers in the pineal organ is not yet fully elucidated, it is evident in the following findings that they derived from the brain: 1) Peptide immunoreactive cells have not been detected in the dog pineal organ. Although Pêvet et al. (1980) and Piekut and Knigge (1982) reported the existence of LHRH-like cells in the rat pineal organ, they may possibly not show nervous elements. 2) After removing the superior cervical ganglia, no changes have been recognized in the distribution of peptidergic fibers in the dog pineal organ. 3) All kinds of peptidergic nerve fibers observed in this organ have been detected in the epithalamic regions at the same time. In addition, the distribution density of the peptidergic fibers within the organ is higher in the proximal part and becomes lower toward the distal part. 4) A direct nervous connection between the brain and the pineal organ in mammals has been proved by morphological and physiological studies (morphological studies: Pfister et al., 1978, Rønnekleiv and Møller, 1979; physiological studies: Rønnekleiv et al., 1978, 1979, Semm and Vollrath, 1978, 1979). Buijs and Pêvet (1980) and Nürnberg and Körf (1981) assumed that pinealopetal vasopressin and oxytocin fibers originated in the paraventricular nucleus.

In two types of peptidergic nerve fibers, thick fibers with large varicosities may be large branches of "en passant" type and thin type fibers with small varicosities may be terminal branches.

Clementi et al. (1965) and Palkovits (1965) reported, with regard to the correlation between the pineal organ and the adrenal cortex, that this organ was involved in the regulation of water- and electrolyte-balance. Buijs and Pêvet (1980) supposed that this function owed to the hormonal effect of vasopressin detected in the organ. On the other hand, Nürnberg and Körf (1981) conceived that the pineal vasopressin and oxytocin fibers were concerned in short-loop feedback information from the neuroendocrine system to the pineal organ. Uddman et al. (1980) assumed that the VIP played as
the neurotransmitter to the blood vessels and to the glandular cells. Thus, regarding the action of the peptides in this organ two significant functions, i.e., hormonal and neurotransmissional (or neuromodulatory), are considered. In addition to proving the functional connection between the brain and the pineal organ by physiological studies (Dafny et al., 1975; McClung and Dafny, 1975; Dafny, 1977; Ronneklev et al., 1980), Schneider et al. (1981) demonstrated many myelinated and unmyelinated fibers, nerve cells and synapses linking to unmyelinated central fibers with intrapineal sympathetic fibers and with processes of intrapineal nerve cells or pinealocytes in the guinea pig. The same morphological features were also demonstrated in the proximal part of the dog pineal organ by electron microscopical observation in this study (unpublished data).

These findings may suggest that various kinds of central input exist in the pineal organ and they not only transmit the information to each other through the synapses but directly influence the pinealocytes. In such a situation it can be sufficiently assumed that peptides contained in the peptidergic nerve fibers in the pineal organ might act as neurotransmitters or neuromodulators. If this hypothesis is provable, it is presumable that the input from the hypothalamus reaches the pineal organ not only by the long pathway through the superior cervical ganglion but also by a short and direct pathway. Peptidergic fibers demonstrated by immunohistochemistry may represent a part of such a short circuit input.

The functional significance of the pinealopetal central nerve fibers remains for further intensive studies.

REFERENCES

Bargmann, W.: Neurosekretion und hypothalamisch-hypophysäres System. Verh. Anat. Ges. 51: 30–45 (1954).
Barry, J.: Les voies extra-hypophysaires de la neurosecréction diencéphalique. Assoc. Anat. 89: 264–279 (1956).
Buijs, R. M. and P. Pévet: Vasopressin- and oxytocin-containing fibers in the pineal gland and subcommissural organ of the rat. Cell Tiss. Res. 205: 11–17 (1980).
Clementi, F., F. Fraschini, E. Müller and A. Zanoboni: The pineal gland and the control of electrolyte balance and of gonadotropic secretion: functional and morphological observations. Progr. Brain Res. 10: 585–603 (1965).
Dafny, N.: Electrophysiological evidence of photic, acoustic, and central input to the pineal body and hypothalamus. Exp. Neurol. 55: 449–457 (1977).
Dafny, N., R. McClung and S. J. Strada: Neurophysiological properties of the pineal body. I. Field potentials. Life Sci. 16: 611–620 (1975).
Korf, H.-W. and U. Wagner: Evidence for a nervous connection between the brain and the pineal organ in the guinea pig. Cell Tiss. Res. 209: 505–510 (1980).
McClung, R. and N. Dafny: Neurophysiological properties of the pineal body. II. Single unit recordings. Life Sci. 16: 621–628 (1975).
Nürnberger, F. and H.-W. Korf: Oxytocin- and vasopressin-immunoreactive nerve fibers in the pineal gland of the hedgehog, Erinaceus europaeus L. Cell Tiss. Res. 220: 87–97 (1981).
Oksche, A.: Survey of the development and comparative morphology of the pineal organ. Progr. Brain Res. 10: 3–29 (1965).
Palkovits, M.: Participation of the epithalamo-epiphysial system in the regulation of water and electrolytes metabolism. Progr. Brain Res. 10: 627–634 (1965).
Pévet, P., I. Ebels, D. F. Swaab, M. T. Mud and A. Arimura: Presence of AVT-, α-MSH-, LH-
Immunohistochemical Peptidergic Fibers in Dog Pineal Organ

RH- and somatostatin-like compounds in the rat pineal gland and their relationship with the UM05R pineal fraction. Cell Tiss. Res. 206: 341–353 (1980).

Pfister, A., J. Muller, P. Leffray, C. Guerillot, E. Vendrely and C. Da Lage: Investigation on a possible extraorthosympathetic innervation of the pineal in the rat and hamster. J. Neural. Transm., Suppl. 13: 390–391 (1978).

Piekut, D. T. and K. M. Knigge: Immunocytochemical analysis of the rat pineal gland using antisera generated against analogs of luteinizing hormone-releasing hormone (LHRH). J. Histochem. Cytochem. 30: 106–110 (1982).

Rønnekleiv, O. K. and M. Møller: Brain-pineal nervous connection in the rat: an ultrastructure study following habenular lesion. Exp. Brain Res. 37: 551–562 (1979).

Rønnekleiv, O. K., M. J. Kelly, M. Møller and W. Wuttke: Electrophysiological and morphological evidence of direct central innervation of the pineal gland. Pflügers Arch. Eur. J. Physiol. Suppl. 373: 54 (1978).

Rønnekleiv, O. K., M. J. Kelly and W. Wuttke: Two excitable neuronal elements in the pineal gland of the rat: further evidence for a habenulo-pineal pathway. Soc. Neurosci. 9th Annu. Meet. Abstr.: 1551 (1979).

———: Single unit recording in the rat pineal gland: evidence for habenulo-pineal neural connections. Exp. Brain Res. 39: 187–193 (1980).

Schneider, T., P. Semm and L. Vollrath: Ultrastructural observation on the central innervation of the guinea-pig pineal gland. Cell Tiss. Res. 220: 41–49 (1981).

Semm, P. and L. Vollrath: Electrophysiological properties of single cells of the guinea pig epiphysis cerebri. Pflügers Arch. Eur. J. Physiol., Suppl. 373: R55 (1978).

———: Electrophysiology of the guinea pig pineal organ: sympathetically influenced cells responding differently to light and darkness. Neurosci. Lett. 12: 93–96 (1979).

Suomalainen, P.: Stress and neurosecretion in the hibernating hedgehog. Dall Museum Comp. Zool. Harvard Coll. 124: 271–283 (1960).

Uddman, R., J. Almets, R. Hakanson, I. Loren and F. Sundler: Vasoactive intestinal peptide (VIP) occurs in nerves of the pineal gland. Experientia 36: 1119–1120 (1980).