Immunohistochemical Studies on Sympathetic and Non-sympathetic Nerve Fibers and Neuronal Cell Bodies in the Pineal Gland of Cotton Rats, *Sigmodon hispidus*

Shoji MATSUSHIMA¹, Yuko SAKAI¹, Yoshiki HIRA¹, Yukio OOMORI¹ and Shigeo DAIKOKU²

Department of Anatomy¹, Asahikawa Medical College, Asahikawa; and Tokushima Research Institute², Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan

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Summary. Immunohistochemistry revealed the presence of tyrosine hydroxylase (TH)-, neuropeptide Y (NPY)-, calcitonin gene-related peptide (CGRP)- and substance P (SP)-immunoreactive nerve fibers and SP-immunoreactive neuronal cell bodies in the pineal gland of the cotton rat (*Sigmodon hispidus*). Abundant TH- and NPY-immunoreactive fibers were distributed evenly throughout the gland; less numerous CGRP- and SP-immunoreactive fibers were distributed in the superficial pineal and the stalk, but were scarce in the deep pineal. All the immunoreactive fibers were usually found around blood vessels. Since TH- and NPY-immunoreactive fibers in various pineal regions disappeared completely with superior cervical ganglionectomy, these fibers are all considered postganglionic sympathetic fibers. Intrapineal CGRP- or SP-immunoreactive fibers decreased considerably in number following superior cervical ganglionectomy, suggesting that some sympathetic fibers contain CGRP or SP. Bilateral bundles of nerve fibers under the transverse sinuses, corresponding to the nervi conarii, contained TH-, NPY-, CGRP- and SP-immunoreactive fibers, which continued into those distributed in the pineal capsule. In the nervi conarii, fibers immunoreactive for TH and NPY disappeared after superior cervical ganglionectomy, but those immunoreactive for CGRP and SP persisted. Thus, non-sympathetic, CGRP- and SP-immunoreactive fibers, together with sympathetic fibers, are presumed to enter the gland by way of the nervi conarii. Neuronal cell bodies, containing SP-like immunoreactivity and being possibly parasympathetic in nature, occurred occasionally in the superficial pineal.

It has long been known that the pineal gland of mammalian species is innervated by three kinds of nerve fibers: peripheral sympathetic, parasympathetic, and central commissural (Vollrath, 1981; Pévet, 1983). Sympathetic fibers have been found to originate from the perikarya in the superior cervical ganglia (Kappers, 1960; Bowers et al., 1984; Reuss and Moore, 1989); however, the origin of the other fibers is not precisely understood. Recent immunohistochemical and retrograde tracing studies have demonstrated the existence of non-sympathetic, pinealopetal nerve fibers containing a variety of peptides as well as the origins of some of these peptidergic fibers in mammals (Korf and Möller, 1985; Möller et al., 1987; Möller, 1992). However, such studies have been conducted on a limited number of species. Earlier observations on the pinealopetal fibers in many mammals deserve reexamination with the aid of modern immunohistochemical and tracing techniques.

Our recent light microscopic study using preparations stained with luxol fast blue and cresyl violet revealed that the pineal gland of cotton rats (*Sigmodon hispidus*) contained, unlike other rodents, abundant myelinated fibers and nerve cells (Matsushima et al., 1991). Myelinated fibers in the pineal gland of this animal persisted following superior cervical ganglionectomy (Karasek et al., 1983a). Myelinated fibers in the pineal gland of this animal persisted following superior cervical ganglionectomy (Karasek et al., 1983a). In a preliminary electron microscopic study of the pineal gland of ganglionectomized cotton rats, we found various types of nerve cells containing granulated vesicles varying from 90 to 140 nm in diameter, and nerve fibers packed with granulated vesicles 100 nm in diameter together with non-granulated vesicles 50 nm in diameter. These findings suggest the existence of peptides in nerve cells and non-sympathetic nerve fibers: peripheral sympathetic, parasympathetic, and central commissural (Vollrath, 1981; Pévet, 1983). Sympathetic fibers have been found to originate from the perikarya in the superior cervical ganglia (Kappers, 1960; Bowers et al., 1984; Reuss and Moore, 1989); however, the origin of the other fibers is not precisely understood. Recent immunohistochemical and retrograde tracing studies have demonstrated the existence of non-sympathetic, pinealopetal nerve fibers containing a variety of peptides as well as the origins of some of these peptidergic fibers in mammals (Korf and Möller, 1985; Möller et al., 1987; Möller, 1992). However, such studies have been conducted on a limited number of species. Earlier observations on the pinealopetal fibers in many mammals deserve reexamination with the aid of modern immunohistochemical and tracing techniques.

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fibers in the pineal gland of cotton rats. Thus, the present immunohistochemical investigation was undertaken to identify peptides contained in nerve cells and non-sympathetic nerve fibers, and to determine the distribution of these structures in the pineal gland of unoperated and superior cervical ganglionectomized cotton rats. In the present study, calcitonin gene-related peptide (CGRP) and substance P (SP) were selected among various peptides hitherto examined.

It has been reported that the pineal gland of cotton rats contains especially numerous sympathetic fibers as compared to that of other rodent species (Karasek et al., 1983b); however, their distribution in relation to various pineal portions has not been examined. The present study thus also attempted to demonstrate sympathetic fibers immunohistochemically using antisera against tyrosine hydroxylase (TH) or neuropeptide Y (NPY), and to determine their distribution in each pineal portion. Based on the results obtained from sympathetic and non-sympathetic fibers, the distribution of both fibers was compared. In addition, whether or not sympathetic fibers are immunoreactive to anti-CGRP or anti-SP was investigated by comparing the distribution of these fibers between unoperated and ganglionectomized animals.

MATERIALS AND METHODS

Twenty male cotton rats (Sigmodon hispidus) from our breeding colony, 12 unoperated and 8 superior cervical ganglionectomized, were used at the ages of 196-243 days (85-136 g body weight). The animals were kept under conditions of controlled lighting (LD14:10; lights on at 0600 h) and temperature (23 ± 2°C), and given food and water ad libitum. Light was provided by cool-white fluorescent lamps, with the intensity at the bottom of the cages being approximately 20 lux. The animals were housed in groups of two to five in a clear plastic cage, 22(w)×39(l)×18 cm (h).

The animals were killed at the middle of the light period (1300 h) between June and October. The animals were anesthetized with sodium pentobarbital (70 mg/kg, i.p.), and perfused through the left cardiac ventricle with phosphate-buffered saline containing heparin (4,000 IU/kg), followed by ice-cold Bouin's fixative without acetic acid. The pineal glands together with the surrounding brain tissue and the covering dura were removed and fixed in the same fixative for 20 h at 4°C, dehydrated in a graded series of ethanol, and embedded in paraffin in vacuo. Serial sagittal sections were prepared at a thickness of 6 μm.

Bilateral superior cervical ganglionectomy was performed under ketamine hydrochloride (15 mg/kg, i.m.) and hexobarbital (55 mg/kg, i.p.) anesthesia 1 week prior to sacrifice. Ptosis was used to check the completeness of the operation.

The immunostaining was performed with the antisera raised in rabbits against TH (Chemicon International), NPY (UCB Bioproducts), CGRP (Cambridge Research Biochemicals) or SP using Vectastain Elite ABC kit (Vector). The SP antiserum used in this study was raised by Tsuruo et al., and its characteristics have been described previously (Tsuruo et al., 1983). Sections were pretreated in 3% H₂O₂, preincubated in 1.5% normal goat serum for 30 min at room temperature, and then incubated at 4°C overnight in the antiserum against TH (diluted 1:5,000), NPY (diluted 1:3,000), CGRP (diluted 1:20,000) or SP (diluted 1:20,000). Sections were incubated with biotinylated anti-rabbit IgG (diluted 1:200) for 1 h at 32°C and then with avidin-biotin-peroxidase complex (diluted 1:25) for 1 h at 32°C. Finally, sections were reacted with 0.01% diaminobenzidine tetrahydrochloride and 0.01% H₂O₂ in 0.05 M Tris-HCl buffer (pH 7.2) for 4 min at 37°C. Sections were counterstained with veronal acetate-buffered 1% methyl green (pH 4.0).

All the serial sections of the pineal gland and adjacent structures from 5 unoperated and 4 ganglionectomized animals were stained with anti-CGRP or anti-SP. In order to examine the relation between CGRP and SP nerve fibers, alternate sections from 1 unoperated and 2 ganglionectomized animals were stained with anti-CGRP and anti-SP. In addition, each set of 3 serial sections from 1 ganglionectomized animal was stained successively with anti-CGRP, hematoxylin and eosin and anti-SP in order to examine the localization of CGRP and SP nerve fibers in the pineal gland and adjacent structures. The distribution of sympathetic fibers was examined in 4 unoperated animals using all the serial sections stained with anti-TH, alternate sections stained with anti-TH and anti-NPY, and sets of 3 serial sections stained successively with anti-TH, hematoxylin and eosin and anti-NPY. The relation between CGRP and TH nerve fibers was examined in 2 unoperated animals using alternate sections stained with anti-CGRP and anti-TH. To examine the effect of ganglionectomy on sympathetic fibers, alternate sections from 1 ganglionectomized animal were stained with anti-TH and anti-NPY.

Camera lucida drawings of nerve fibers immunoreactive to different antisera in every 10th section of the pineal gland and habenular and posterior commissures were made at a magnification of ×400 in order to compare their distribution. In addition, all the serial sections from the pineal glands of each group...
of 3 unoperated and ganglionectomized animals were examined to determine the distribution of cell bodies showing SP-like immunoreactivity; the profiles of the pineal gland were drawn, and the cell bodies in the profiles were plotted. Care was taken not to plot the same cell body twice.

The staining specificity of the TH, NPY or CGRP antiserum was tested by replacement of the antiserum by normal rabbit serum or phosphate-buffered saline. The NPY or CGRP antiserum was also tested for specificity by preabsorption of the antiserum with synthetic NPY (Peptide Institute, 1 µg/ml) or CGRP (Peptide Institute, 1 µg/ml). In these experiments, no specific immunostaining was detected in nerve fibers. The specificity of the SP antiserum has been reported elsewhere (TsURUO et al., 1983).

RESULTS

Examination of serial sagittal sections of the brain and the covering dura of unoperated animals revealed cross-sectioned, bilateral bundles consisting of tyrosine hydroxylase (TH)- and neuropeptide Y (NPY)-immunoreactive nerve fibers under the floor of the transverse sinuses and the confluens sinuum (Fig. 1). Nerve bundles on both sides could be traced to a single bundle running longitudinally along the lateral margin of the opening of the great cerebral vein into the confluens (Fig. 2), and farther to bundles distributed in the capsule of the distal region and the ventral surface of the superficial pineal (Fig. 3). TH- and NPY-immunoreactive fibers were found in similar locations in bundles in the dura mater (Figs. 4, 5) and the capsule, where NPY fibers usually stained less intensely than TH fibers (Fig. 4 vs. Fig. 5). The bundles under the transverse sinuses and the confluens sinuum were oval in cross section and measured about 26 and 13 µm in long and short diameters, respectively. The bundles appeared to contain some fibers, which remained unstained with anti-TH and anti-NPY (Figs. 4, 5). So far as sagittal sections of the dural tissue adjacent to the pineal gland were examined, no TH- or NPY-immunoreactive fibers were observed in the dura mater except the sites mentioned above. There were no TH- or NPY-im-

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**Figs. 1–3.** Representative serial sections showing nerve bundles immunoreactive for TH (arrows) in the dura mater (D) and the capsule (C) covering the distal pineal portion (P) of an unoperated animal. Sections in Fig. 1 and Figs. 2 and 3 are obtained from the right and left sides of the brain, respectively; the section in Fig. 2 is cut at a somewhat more lateral level than that in Fig. 3. TS transverse sinus, CS confluens sinuum, GCV great cerebral vein, asterisk opening of the great cerebral vein into the confluens. × 450

**Fig. 4 and 5.** Nerve bundle (enclosed by arrowheads) under the confluens sinuum (CS) of an unoperated animal in adjacent sections stained with anti-TH (Fig. 4) or anti-NPY (Fig. 5). D dura mater. × 900
munoreactive fibers in the wall or the great cerebral vein. TH-immunoreactive fibers were absent in the habenular and posterior commissures, whereas there were some fibers showing NPY-like immunoreactivity in these commissures (Fig. 8).

In unoperated animals, all pineal regions had a dense innervation of TH- and NPY-immunoreactive fibers, which usually appeared as single fibers with varicosities (Figs. 6, 7). These fibers meandered in the superficial pineal (Fig. 6), but assumed a straight course in the stalk, lying in association with blood vessels. TH- and NPY-immunoreactive fibers were scarce among parenchymal cells in the superficial pineal. By contrast, in the deep pineal, TH- and NPY-immunoreactive fibers were localized not only around blood vessels, but also among parenchymal cells (Fig. 7). In all pineal regions, the distribution and the density of TH-containing fibers were similar to those of NPY-containing fibers, and the density of both fibers seemed to be uniform throughout the gland (Fig. 8).

Bilateral removal of the superior cervical ganglia resulted in the complete disappearance of TH- and NPY-immunoreactive fibers from the dura mater, the pineal capsule and the pineal gland. NPY-containing fibers in the habenular and posterior commissures remained intact following superior cervical ganglionectomy.

The localization and features of CGRP- and SP-immunoreactive fibers in the pineal gland and adjacent structures were similar in unoperated and ganglionectomized animals. In the dura mater and the pineal capsule, CGRP fibers were usually found as bundles, and were more widely distributed than TH.

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**Fig. 6.** NPY-immunoreactive fibers in the superficial pineal of an unoperated animal. *arrows* Blood vessels. ×450

**Fig. 7.** NPY-immunoreactive fibers in the deep pineal of an unoperated animal. *arrows* Blood vessels, *asterisk* third ventricle, *HC* habenular commissure. ×450
and NPY fibers. CGRP-immunoreactive fibers were numerous in the floor of the superior sagittal sinus, the transverse sinuses and the confluens sinuum (Figs. 9-11), especially around the opening of the great cerebral vein into the confluens (Figs. 9, 11), and in the capsule surrounding the distal region and the ventral surface of the superficial pineal (Figs. 9, 12). Examination of serial sections indicated that bilateral, thick bundles of CGRP-immunoreactive fibers under the floor of the transverse sinuses and the confluens sinuum (Fig. 10) could be followed to a single bundle along the lateral margin of the opening of the great cerebral vein into the confluens (Fig. 11), and to bundles in the pineal capsule (Fig. 12). In the dura mater and the pineal capsule, the localization of SP-immunoreactive fibers was very similar to that of CGRP-immunoreactive fibers, though SP fibers were less numerous than CGRP fibers (Fig. 12 vs. Fig. 13). Both CGRP- and SP-immunoreactive fibers occurred also in the wall of the great cerebral vein (Figs. 12, 13). Serial sections stained with different antisera showed that, in unoperated animals, CGRP- and SP-immunoreactive fibers usually occupied a position different from that of TH- and NPY-immunoreactive fibers in the bilateral bundles under the transverse sinuses (Fig. 14 vs. Fig. 15).

CGRP-immunoreactive fibers were found scattered evenly throughout the superficial pineal (Fig. 9); these fibers usually wound as single varicose fibers along blood vessels (Fig. 16). Intraparenchymal CGRP-containing fibers were scarcely found. In the superficial pineal, SP-immunoreactive fibers were scattered as single varicose fibers in the peripheral area, but were scarce in the central area. In the stalk, abundant CGRP-immunoreactive fibers and less numerous SP-immunoreactive fibers took a straight course along blood vessels. Both fibers were scarce in the deep pineal, and no CGRP- and SP-containing fibers were observed in the habenular or posterior commissures (Fig. 17).

Bilateral superior cervical ganglionectomy resulted in a marked decrease in the number of CGRP- and SP-immunoreactive fibers from the superficial pineal and the stalk, though considerable numbers of these fibers persisted (Fig. 18). CGRP- and SP-containing fibers in the dura mater, the pineal capsule and the wall of the great cerebral vein appeared to remain unchanged in ganglionectomized animals.

Cell bodies with SP-like immunoreactivity were scattered in the superficial pineal. Cell bodies varied in size and staining (Figs. 19, 20). Occasionally, SP-immunoreactive processes occurred around cell bodies (Fig. 20). These processes appeared to arise from cell bodies, and occasionally could be traced for a considerable distance. Figure 21 shows the distribution of all SP-immunoreactive cell bodies in the pineal glands of 3 unoperated animals. As seen in this figure, SP-containing cell bodies ranged in number from 12 to 20, and the majority of the cell bodies were localized in the ventral area of the superficial pineal.
The long diameter of a total of 50 cell bodies immunoreactive for SP was $14.4 \pm 3.2 \mu m$. In ganglionectomized animals, morphological features of SP-immunoreactive cell bodies were similar to those in unoperated animals. The long diameter of a total of 25 cell bodies immunoreactive for SP in 2 ganglionectomized animals was $14.8 \pm 3.5 \mu m$.

Cell bodies immunoreactive for TH, NPY or CGRP were not detected in the materials used in the present study.

**DISCUSSION**

The pineal gland of rodents is composed of the superficial pineal lying under the confluens sinuum, the deep...
pineal interposed between the habenular and posterior commissures, and the stalk connecting the two portions (Vollrath, 1981). Previous studies using formaldehyde- or glyoxylic acid-induced fluorescence methods have found that catecholaminergic fibers are abundantly distributed in the superficial and deep pineal of the rat (Björklund et al., 1972; Wiklund, 1974), golden hamster (Reiter and Hedlund, 1976) and gerbil (Möller et al., 1979), and that these fibers disappear from both pineal portions following superior cervical ganglionectomy.

Recent immunohistochemical studies have demonstrated fibers immunoreactive for TH or NPY in the pineal glands of some rodents (Schon et al., 1985; Schröder, 1986; Shiotsuki et al., 1986); these fibers were eliminated after superior cervical ganglionectomy (Schon et al., 1985; Shiotsuki et al., 1989). In the above studies, however, the pineal regions examined were never indicated. The distribution of TH- or NPY-immunoreactive fibers in relation to pineal regions was first examined in the rat by Zhang et al. (1991), who found that fibers immunoreactive for TH or NPY were abundant in both the superficial and deep pineal, and that TH or NPY fibers almost disappeared from the superficial pineal but remained in the deep pineal following superior cervical ganglionectomy. Thus, these authors have suggested that TH- or NPY-containing fibers in the deep pineal of the rat originate from the central nervous system. The pineal gland of the cotton rat also consists of three portions (Matsushima et al., 1991). The present study revealed that TH- or NPY-immunoreactive fibers were distributed evenly throughout the organ, and that these fibers disappeared completely with superior cervical ganglionectomy. This observation agrees with previous fluorescence histochemical data obtained from other rodents, including the rat (Björklund et al., 1972; Wiklund, 1974; Reiter and Hedlund, 1976; Möller et al., 1979), but not with the above mentioned, immunohistochemical results from the rat (Zhang et al., 1991). The reason for this discrepancy is not known.

Topographical relations of sympathetic fibers to blood vessels or parenchymal cells in the mammalian pineal gland have been examined by fluorescent histochemical (Owman, 1965; Möller and Van Veen, 1981) and electron microscopic studies (Matsushima and Reiter, 1975, 1977; Karasek et al., 1983b; see also Vollrath, 1981; Korf and Möller, 1984, for review). Sympathetic fibers usually run around blood vessels, though in some species these fibers are also found in the parenchyma. Our electron microscopic study revealed that intraparenchymal sympathetic fibers in the pineal gland of cotton rats lay close to the so-called interstitial cells and astrocytes, but not to pinealocytes (unpublished observations). Interstitial cells and astrocytes were usually arranged in a
Fig. 17. Distribution of CGRP-immunoreactive fibers on a sagittal plane of an unoperated animal. CGRP-containing fibers in every 10th section have been drawn. Numbers over each pineal profile represent numbers of serial sections. Shaded areas indicate habenular (HC) and posterior (PC) commissures.

Fig. 18. Distribution of CGRP-immunoreactive (A, B) and SP-immunoreactive (C, D) fibers on a sagittal plane of unoperated animals (A, C) and a ganglionectomized animal (B, D). All the CGRP-containing fibers in Fig. 17 and SP-containing fibers in every 10th section from a different animal have been superimposed to depict Fig. 18 A and C, respectively. Likewise, CGRP-containing fibers in every 10th section and SP-containing fibers in adjacent sections from an animal have been superimposed to depict Fig. 18 B and D, respectively. Shaded areas indicate habenular (HC) and posterior (PC) commissures.
single layer around pericapillary spaces in the superficial pineal. Unlike the superficial pineal, the deep pineal contained more astrocytes but no interstitial cells. In this region, groups of cell bodies of astrocytes and their processes surrounded pericapillary spaces; in addition, overlapping astrocytic processes were often interposed among pinealocytes. Also, sympathetic fibers were scattered among astrocytic processes. These results may explain the observed differences in the distribution of TH- or NPY-immunoreactive fibers between the superficial and deep pineal of the cotton rat. A close topographical relation between sympathetic fibers and interstitial cells has also been found in the pineal gland of some rodents (Wolfe, 1965; Matsushima and Reiter, 1975). These morphological data suggest a functional correlation between sympathetic fibers and interstitial cells or astrocytes in the mammalian pineal gland.

In the pineal gland of the cotton rat, fibers immunoreactive for CGRP or SP, which survive after superior cervical ganglionectomy, distribute themselves abundantly in the distal region, but decrease in number toward the proximal region. The pineal gland of this animal, unlike other rodents, contains abundant myelinated fibers (Matsushima et al., 1991). Myelinated fibers, though less numerous than CGRP- or SP-immunoreactive fibers, also decrease in number toward proximal levels. Thus, it is assumed that CGRP- or SP-immunoreactive fibers and myelinated fibers belong to the same group of fibers. Examination of serial sections shows that bilateral bundles of myelinated fibers running under the transverse sinuses continue into myelinated fibers distributed in the capsule of the distal portion of the superficial pineal of the cotton rat (Matsushima et al., 1991). Their courses in the dural tissue lead to myelinated fibers being considered to form part of the nersi conarii. Since myelinated fibers are scarce in the deep pineal, these fibers may not originate from commissural fibers (Matsushima et al., 1991). SP-immunoreactive fibers, which are sparsely distributed in the pineal gland of the rat, are believed to originate from the central nervous system (Ronneklev and Kelly, 1984). In the cotton rat, fibers immunoreactive for CGRP or SP are sparse in the deep pineal and absent in the habenular and posterior commissures. Thus, CGRP or SP fibers may not be derived from the central nervous system. The present results suggest that, in the cotton rat, the nersi conarii are composed of TH-, NPY-, CGRP- and SP-immunoreactive fibers which continue into those distributed in the pineal capsule. After superior cervical ganglionectomy, TH and NPY fibers were lost, but CGRP and SP fibers persisted in the nersi conarii. Thus, non-sympathetic, CGRP- and SP-immunoreactive fibers, together with sympathetic fibers, are considered to enter the gland by way of the nersi conarii.

Examination of adjacent sections stained with antibodies against CGRP and SP suggests the coexistence of these peptides in the same fibers in the pineal gland of cotton rats. Immunohistochemical studies of the dura mater have demonstrated abundant fibers immunoreactive for CGRP or SP in the wall of the superior sagittal and transverse sinuses in the rat (Keller and Marfurt, 1991). Combined retrograde tracing and immunohistochemical studies have indicated that these fibers originate from the trigeminal ganglia (O’Connor and Van Der Kooy, 1988; Uddman et al., 1989). Tracing and immunohistochemical techniques have revealed that CGRP- and SP-immunoreactive fibers in the pineal gland of the rat (Reuss et al., 1992) and gerbil (Shiotani et al., 1986) arise from the trigeminal ganglia. These findings, in combination with the present results, suggest...
the trigeminal ganglia as the most probable source of CGRP- and SP-immunoreactive fibers in the pineal gland of the cotton rat. Retrograde tracing experiments are in progress to prove this assumption.

In the pineal gland of cotton rats, some of the sympathetic fibers are presumed to contain CGRP or SP because fibers immunoreactive for these peptides decrease considerably in number following superior cervical ganglionectomy. The present study is the first to describe the presence of CGRP or SP in pineal sympathetic fibers in mammals. Recent immunohistochemical studies have demonstrated that neuronal cell bodies in the superior cervical ganglia in some mammals contain CGRP or SP (ARIANO and KENNY, 1985; KUMMER and HEYM, 1988; SCHMITT et al., 1988; BAFFI et al., 1992). As shown in the present study, only the sympathetic fibers, which are distributed in the region richly supplied with non-sympathetic, CGRP- or SP-immunoreactive fibers, were reactive for CGRP or SP in the cotton rat. Thus, non-sympathetic, CGRP or SP fibers are closely related to sympathetic fibers. Details on this relation will be considered in a future study.

The nature of nerve cells in the mammalian pineal gland is still unknown (for review see KORF and MÖLLER, 1984). Immunohistochemical studies are expected to provide new information on intrapineal neurons. Recent immunohistochemical observations have revealed the presence of neurons or neuron-like cells immunoreactive for enkephalin (MOORE and SIBONY, 1988; SCHRÖDER et al., 1988) and TH or dopamine-β-hydroxylase (SCHRÖDER and VOLLRATH, 1985; JIN et al., 1988; JENGELESKI et al., 1989) in some rodents and the human. In the cotton rat, the superficial pineal contains small numbers of SP-immunoreactive cell bodies, which are usually situated in its ventral area. The long diameter of the SP-containing cell bodies is identical with that of nerve cells in cresyl-violet-stained sections of the superficial pineal of this animal (MATSUSHIMA et al., 1991). Intrapineal neurons in cotton rats are mainly localized in the ventral area of the distal pineal region (MATSUSHIMA et al., 1991). Thus, cell bodies immunoreactive for SP may not represent pinealocytes but nerve cells. It is possible that some SP-immunoreactive fibers in the pineal gland of cotton rats originate from SP neurons. The presence of intrapineal neurons immunoreactive for SP in mammals has not hitherto been reported. Intrapineal neurons in the monkey (KENNY, 1961) and rabbit (ROMIJN, 1975) have been tentatively considered to represent parasympathetic neurons. A recent immunohistochemical study has demonstrated the presence of neurons immunoreactive for choline acetyltransferase (ChAT) in the bovine pineal gland (PHANSUWAN-PUJITO et al., 1991). Since neurons in parasympathetic ganglia contain SP in addition to other neuropeptides including VIP (HARDEBO et al., 1992), SP-immunoreactive cell bodies in the superficial pineal of cotton rats may belong to the parasympathetic nervous system. However, this assumption should be verified by immunohistochemical demonstration of ChAT and VIP in intrapineal neurons of this animal. This will be the subject of a future study.

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