Descending Projections from the Paraventricular Hypothalamic Nucleus to the Spinal Cord: Anterograde Tracing Study in the Rat

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Summary: We investigated the direct projections from the paraventricular hypothalamic nucleus (PVH) to the spinal cord. When *Phaseolus vulgaris* leucoagglutinin (PHA-L) was injected into the PVH, labeled descending fibers were observed running bilaterally through three pathways. The first pathway ran into the dorsal longitudinal fasciculus and projected to the central gray matter, Edinger-Westphal nucleus, pedunculo-pontine tegmental nucleus, nucleus of the locus ceruleus and parabrachial nucleus. The second and third pathways coursed through the medial forebrain bundle, ventral tegmental area, and ventral part of the medulla oblongata. At the medulla oblongata, the second pathway curved dorsolaterally and joined Rexed's lamina V of C1 after giving many projections to the nucleus ambiguus, nucleus of the solitary tract, dorsal motor nucleus of the vagus, and a few to the area postrema. The fibers descended through lamina V until C5, and coursed through lamina I from C6 to the upper coccygeal segments. They gave collateral projections to lamina I from the cervical through the upper coccygeal segments. The third pathway coursed laterally and descended through the lateral funiculus after giving projections to the lateral reticular nucleus and the marginal layer of the spinal trigeminal nucleus. These fibers gave off many projections to the intermediolateral cell column of the thoracic cord and the sacral parasympathetic nucleus. Lamina X received many projections from the fibers of the lateral funiculus at C5 through the b-segment of sacral spinal cord.

These results indicate that the PVH may integrate directly with the medullary and spinal autonomic regulatory nuclei, including the vagus complex, sympathetic intermediolateral cell column, laminae I and X, and sacral parasympathetic nucleus.

The paraventricular hypothalamic nucleus (PVH) is composed of several functionally distinct subnuclei that affect both endocrine and autonomic responses (Swanson, 1977; Buijs, 1978; Nilaver *et al.*, 1978; Armstrong *et al.*, 1980; Pittman *et al.*, 1981). The magnocellular nucleus of the PVH sends relatively short axons to the endocrine system of the pituitary gland (Sherlock *et al.*, 1975; Swanson and Kuypers, 1980; Wiegand and Price, 1980; Kiss *et al.*, 1983). The parvocellular nucleus of the PVH sends axons not only to the median eminence but to various nuclei in the brainstem and spinal cord, such as the parabrachial nucleus, the nucleus of the locus ceruleus, the dorsal motor nucleus of the vagus, the nucleus of the solitary tract, the intermediolateral cell column and the sacral parasympathetic nucleus (Hancock, 1976; Saper *et al.*, 1976; Swanson, 1977; Cedarbaum and Aghajanian, 1978; Ono *et al.*, 1978; Hosoya and Matsushita, 1979; Swanson and Mckellar,

1979; Holstege, 1987).

These nuclei regulate autonomic charges occurring in the sympathetic and parasympathetic outflow system. The PVH receives general visceral and autonomic information from the bed nucleus of the stria terminalis, the septal nucleus, the preoptic nuclei, the lateral hypothalamic nucleus, the amygdala, the parabrachial nucleus, and the nucleus of the solitary tract (Silverman *et al.*, 1981; Sawchenko and Swanson, 1983; Fulwiler and Saper, 1984; Cunningham and Sawchenko, 1988; Simerly and Swanson, 1988; Ter Horst *et al.*, 1989; Cullinan *et al.*, 1993). For these reasons, the PVH is considered one of the major forebrain autonomic structures.

Many retrograde tracing studies using horseradish peroxidase (HRP) or fluorescent dyes have revealed direct projections of the PVH to the cervical, thoracic and lumbar spinal cord (Ono *et al.*, 1978; Hosoya

and Matsushita, 1979; Swanson and Kuypers, 1980; Tucker and Saper, 1985). These studies, however, checked only specific segments at different levels in the spinal cord. Luiten *et al.* (1985) showed, in an anterograde tracing study, that there were two descending pathways from the PVH in the brainstem. However, they did not adequately describe the descending fibers in the lower medulla and spinal cord. Swanson and Kuypers (1980) suggested that most of the PVH neurons projecting to the spinal cord were different from those projecting to the vagal nuclear complex.

The present study was attempted to investigate the route of the PVH descending fibers and the projection region in the medulla and in each segment of the spinal cord, using the anterograde tracing method of *Phaseolus vulgaris* leucoagglutinin (PHA-L).

Materials and Methods

Twenty two male Wistar rats were used in this study. All surgical procedures were carried out under sodium pentobarbital (30 mg/kg, i.p.) anesthesia.

For the anterograde tracing studies, restricted PHA-L injections were made into the PVH. A fine tip glass pipet (diameter 10–12 μ m) was filled with 2.5% PHA-L (Seikagaku, Tokyo) in 0.1 M phosphate buffer at pH 7.4. The tracer was injected iontophoretically with a current of 10–20 μ A, applied in an alternative mode (5 seconds on, 5 seconds off). After 10 to 14 days, the animals were perfused with 200 ml saline, followed by 200 ml of a solution containing 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer, and then with 500 ml of 4% paraformaldehyde. The brain and all segments of the spinal cord were removed, embedded in an agarose-gelatin mixture, and post-fixed overnight with a 4% paraformaldehyde solution containing 20% sucrose. The brain and spinal cord were cut with a freezing microtome at 40 μ m and collected in a chilled 1% bovine serum solution in 0.1 M Tris-HCl buffer containing 0.1% Triton-X100 and 0.9% sodium chloride at pH 7.4.

Immunohistochemical procedures for PHA-L were carried out according to Gerfen and Sawchenko (1984). Every third section of the brainstem and all segments of the spinal cord was incubated for two days in rabbit anti-PHA-L antibodies (E-Y Labs, dilution 1:800). The primary antibodies were localized by incubation in biotinylated goat anti-rabbit-IgG (Vector, dilution 1:2000), and in a commercially available ABC kit (Vector). The sections were rinsed in Tris-buffer solution containing Triton-X100 between each incubation. We employed the cobalt-glucose oxidase method with DAB of Itoh *et al.*

(1979) to visualize the labeled fibers and terminal-like boutons. After mounting on gelatin-coated slides, the sections were dehydrated, coverslipped and observed under bright or darkfield illuminations. Some sections containing labeled terminal-like boutons were dehydrated with ether-alcohol (1:1) and counterstained with thionin. We followed the terminology of Paxinos and Watson (1986) for the nuclei in the brainstem and the spinal cord, and the terminology of the Swanson and Kuypers (1980) for the subnucleus in the PVH.

Results

The injection of PHA-L was applied precisely to the PVH. The center of injection was distinguished as brown-black deposit. The center of the injection sites for 10 cases are shown in Figure 1. These included the dorsal, medial, lateral and posterior parvocellular divisions, and the posterior magnocellular division (Figs. 1, A, B). All cases of which injection sites included mainly the parvocellular part of PVH (Cases, P25, P26, P31, P33, P35), had descending labeled fibers and terminal-like boutons in the spinal cord (Table). The distribution pattern of the labeled fibers and terminal-like boutons was similar in these cases. But the cases of which injection sites included mainly the magnocellular part of PVH (Cases, P12, P19, P28, P29, P32), had a few labeled descending fibers in the lower brainstem, and no labeled fibers in the spinal cord (Table). Because the parvocellular and magnocellular subnuclei are small, and have complex shapes, we could not show topographical descending projections for these subnuclei. The injection site of P35 was restricted to the medial parvocellular and the ventromedial edge of the posterior magnocellular parts (Figs. 1, A, C). Since the labeled descending fibers were many and conspicuous in case P35, we showed this as a typical case (Fig. 2). The descending fibers from the PVH initially divided into two main pathways, that is a dorsal and a ventral pathways. Many labeled fibers from the injection site ran along the contralateral side through the periventricular gray of the third ventricle, although the density of labeled fibers was greater on the ipsilateral side.

The fibers of the dorsal pathway descended along the periventricular region and entered the dorsal longitudinal fasciculus (Figs. 2, A–E). There were many bilateral fibers and terminal-like boutons in the Edinger-Westphal nucleus, the ventral part of the midbrain central gray matter, the pedunclopontine tegmental nucleus, the dorsal raphe nucleus, the parabrachial nucleus, and the nucleus of the locus ceruleus (Figs 2, C–E). The number of labeled

Table Distribution of anterogradely labeled terminal-like boutons in the midbrain pons, medulla oblongata and spinal cord after PHA-L injection into the paraventricular hypothalamic nucleus.

Nuclei	Parvocellular part					Magnocellular part				
	P25	P26	P31	P33	P35	P12	P19	P28	P29	P32
Midbrain central gray matter	+++	+++	+++	++	+++	+	++	++	+	++
Edinger-Westphal n.	+	+	+	+	+	+	+	+	-	+
Interfascicular n.	+	+	+	+	++	+	+	+	+	+
Rostral linear n.	+	+	+	+	++	+	+	+	+	-
Lateral lemniscus n.	++	+	++	+	+++	+	+	+	-	+
Pedunculopontine tegmental n.	++	++	+	+	+++	+	+	+	+	+
Median raphe n.	+	++	++	+	++	+	+	+	+	+
Dorsal raphe n.	+	++	++	+	++	+	+	+	+	+
Locus ceruleus n.	++	++	++	++	++	+	+	+	+	+
Parabrachial n.	++	++	++	++	++	+	+	+	+	+
Raphe magnus n.	+	+	++	+	++	+	+	+	+	+
Raphe pallidus n.	+	+	++	+	++	+	+	+	+	+
Lateral reticular n.	++	+	++	+	++	-	-	-	-	-
Ambiguous n.	+	+	+	+	+	-	+	+	-	+
Lateral paragigantocellular n.	+	+	+	+	++	+	+	+	-	-
Dorsal motor n. of vagus	+++	++	+++	++	+++	+	+	+	+	+
N. of the solitary tract	+++	++	+++	++	+++	+	+	+	+	+
Area postrema	+	+	+	-	+	-	-	-	-	-
Spinal trigeminal n.	+	+	+	+	++	-	-	-	-	-
Intermediolateral cell column	+	+	++	+	++	-	-	-	-	-
Sacral parasympathetic n.	+	+	++	+	++	-	-	-	-	-
Lamina I of spinal cord	+	+	++	+	++	-	-	-	-	-
Lamina V of spinal cord	+	+	+	+	++	-	-	-	-	-
Lamina X of spinal cord	+	+	+++	+	+++	-	-	-	-	-

-; absence, +; a few labeled terminal-like boutons, ++; many labeled terminal-like boutons, +++; numerous labeled terminal-like boutons.

fibers and terminal-like boutons was slightly less on the contralateral side. There were a few collateral fibers and terminal-like boutons in the superior colliculus, the inferior colliculus, and the dorsal region of the midbrain central gray matter. A few labeled fibers scattered in the pontine reticular formation (Fig. 2, E). Only a few labeled fibers were seen in the surrounding areas of the dorsal tegmental nucleus. The labeled fibers in the dorsal longitudinal fasciculus ended in the dorsal tegmentum of the pons, and no labeled fibers were seen at the level of the facial genu (Figs. 2, E, F).

The fibers of the ventral pathway coursed through the medial forebrain bundle, ventral tegmental area, and along the interpeduncular nucleus. Some fibers branched off from this bundle, ran bilaterally along the dorsolateral aspect of the substantia nigra, and terminated in the nucleus of the lateral lemniscus (Fig. 2, C) and the ventral region of the pedunculopontine tegmental nucleus (Fig. 2, D). The major fibers of the ventral pathway run through the ventrolateral part of the pontine tegmentum and dorsal to the pyramidal tract (Figs. 2, D-H). Throughout their course, these fibers gave off terminal-like

boutons in the nucleus linearis rostralis, the median raphe nucleus, the nucleus raphe magnus, and the nucleus raphe pallidus (Figs. 2, C-G).

At the medulla oblongata, the major fibers coursed through the ventromedial aspect of the superior olivary nucleus, and entered into the lateral paragigantocellular nucleus (Figs. 2, E-G). The fibers diverged bilaterally between the levels of the superior olivary nucleus and the lateral reticular nucleus (Figs. 2, E-H). Those branches ran along the dorsal aspect of the facial nucleus, and entered into the lateral reticular nucleus. A large number of labeled terminal-like boutons were found in the A5 noradrenaline cell group (Fig. 2, E) and the lateral region of the lateral reticular nucleus (Figs. 2, G, H; 3, A, B). A few, but distinctly labeled terminal-like boutons were seen at the ventral surface of the contralateral lateral paragigantocellular nucleus. At the level of the lateral reticular nucleus, the fibers of the ventral pathway again divided into lateral and dorsal bundles (Figs. 2, G, H).

After providing many terminal-like boutons to the lateral part of the lateral reticular nucleus (Fig. 3, B), the fibers of the lateral bundle descended

caudolaterally and passed through the dorsal part of the lateral funiculus of the spinal cord (Figs. 2, H, C1). Some fibers exited laterodorsally and gave terminal-like boutons in the ventral part of the marginal layer of the spinal trigeminal nucleus (Figs. 2, G, H; 3, C). The dorsal bundle turned dorso-medially from the lateral reticular nucleus, traversed the nucleus ambiguus (Fig. 3, A) and the parvocellular reticular nucleus, and entered into the vagal nuclear complex (Fig. 3, E). Heavily labeled fibers and terminal-like boutons were observed bilaterally throughout the nucleus of the solitary tract and the dorsal motor nucleus of the vagus (Figs. 2, H; 3, D). There were a few labeled fibers and terminal-like boutons in the area postrema (Figs. 2, H; 3, D), as well as conspicuously labeled terminal-like boutons in the nucleus ambiguus and the C1 adrenaline cell group (Fig. 3, A). Some fibers branched off laterally and terminated in the dorsal region of the marginal layer of the spinal trigeminal nucleus (Figs. 2, G, H). After projecting to the vagal nuclear complex, fibers of the dorsal bundle turned laterally and entered Rexed's lamina V of the cervical spinal cord (Figs. 3, E, F).

In the spinal cord, fibers of the dorsal bundle descended mainly through lamina V from the level of C1 to C5. The fibers in lamina V diverged dorso-laterally along the medial edge of lamina IV and entered lamina I (Figs. 2, C1, C5) from the level of C1 to C5. Downward from the level of C5, the majority of the fibers descended through lamina I until the rostral half of the coccygeal segment. Only a few fibers remained to run in lamina IV at the thoracic and lumbar segments (Fig. 5, B). The labeled fibers descending through lamina I did not intermingle with the fibers of the lateral funiculus (Fig. 5, A, B). Many fibers and terminal-like boutons were present bilaterally in lamina I in all segments from the cervical to the rostral half of the coccygeal cord (Figs. 2, C1-Co1; 4, A; 5, A, B; 6, C), although they were decreased on the contralateral side. We could not follow the fibers at the level of the caudal half of the coccygeal segment.

Many labeled fibers were observed bilaterally from the cervical through the caudal coccygeal segment in the lateral funiculus (Figs. 2, C1-Co2; 5, A, B; 6, A, C). These fibers were dominant on the ipsilateral side. From the level of C5 to the c-segment of the sacral spinal cord (Sc), the labeled fibers branched medially to enter the gray matter (Figs. 2, C5-Sb). These fibers ran through lamina VII from C5 to T12 (Figs. 2, C5-T8), or through lamina VI from L1 to Sb, and entered lamina X (Figs. 2, L2-Sb). Distinctly labeled fibers and terminal-like boutons were present in lamina X through C5 to the caudal end of the sacral spinal segment, with a

particular large number of terminal-like boutons present from T8 to Sa (Figs. 2, T8-Sa; 4, B; 6, A). There were no labeled fibers running into the lamina I from the lateral funiculus (Fig. 5, A, B; 6, C).

At all levels between T3 and L4, the fibers projecting to lamina X branched off bilaterally and terminated in the intermediolateral cell column (Figs. 2, T3-L2; 4, C). Medium-sized elongated neurons received conspicuous labeled terminal-like boutons (Fig. 4, D). There were fewer terminal-like boutons on the contralateral side. The sacral parasympathetic nucleus received a large number of terminal-like boutons from fibers projecting to lamina X at all levels from L5 to Sb on both sides (Figs. 2, L6-Sb; 6, A). The contralateral sacral parasympathetic nucleus received many terminal-like boutons although there were more on the ipsilateral side (Figs. 2, L6-Sa). Many beaded labeled terminal-like boutons were in close apposition to the medium-sized neurons in the sacral parasympathetic nucleus (Fig. 6, B). Only a few labeled fibers were present in the anterior horn of Sa, and labeled terminal-like boutons were found there (Fig. 2, Sa). Although few in number distinct labeled fibers were observed descending through the lateral funiculus of the coccygeal spinal cords (Fig. 6, C). However, we could not find labeled terminal-like boutons in this area.

Discussion

Our results revealed that the PVH descending projection was composed of three pathways. The first ran through the dorsal longitudinal fasciculus and terminated in the pedunculopontine tegmental nucleus, the parabrachial nucleus and the nucleus of the locus ceruleus. Both the second and the third pathways descended first through the medial fore-brain bundle and the ventral tegmentum. They sent collateral fibers to the nucleus of the lateral lemniscus and the pedunculopontine tegmental nucleus. At the medulla oblongata, the second pathway curved dorsolaterally and descended through lamina V or lamina I through the cervical to the coccygeal spinal cord. The fibers gave a large number of terminal-like boutons at the dorsal motor nucleus of the vagus, the nucleus of the solitary tract, and lamina I at all spinal segments. The third pathway coursed through the ventral medulla, entered the dorsal part of the lateral funiculus and descended to the lower end of the coccygeal spinal cord. These fibers gave a large number of terminal-like boutons at the intermediolateral cell column, lamina X, and the sacral parasympathetic nucleus (Fig. 7).

Retrograde tracing studies reported that the PVH projected directly to the cervical, thoracic and lumbar

spinal cord (Ono *et al.*, 1978; Hosoya and Matsushita, 1979; Hosoya, 1980; Swanson and Kuypers, 1980; Schwanzel-Fukuda *et al.*, 1984; Tucker and Saper, 1985; Wagner and Clemens, 1991). Tucker and Saper (1985) found that there was no topographic organization between segments of the thoracic spinal cord and neuronal cell groups in the PVH. We found that the PVH projected not only to the cervical, thoracic, and lumbar regions, but also to the sacral spinal cord. The spinal projection neurons are mainly present in the parvocellular division. The magnocellular division also sends a few descending fibers to the medulla oblongata. Our results agree with these studies. We found heavy projections bilaterally at the medullary nuclei relating to the autonomic functions, including the dorsal motor nucleus of the vagus, and the nucleus of the solitary tract, and a few to the area postrema and the nucleus ambiguus. These results confirm previous anterograde and retrograde tracing studies (Saper *et al.*, 1976; Swanson and Kuypers, 1980; Hosoya and Matsushita, 1981; Hosoya, 1985; Holstege, 1987). There were also projections at the A5 noradrenaline and C1 adrenergic cell groups. Both A5 and C1 groups project to the sympathetic preganglionic neurons of the thoracic spinal cord (Ross *et al.*, 1981; Goodchild *et al.*, 1984), and show sensitive vasopressor and respiratory activity (McAllen, 1986a, b; Otake *et al.*, 1990; Morrison *et al.*, 1991). Thus, the PVH may regulate sympathetic vasomotor and respiratory activity via these cell groups.

There have been several studies on descending projections from the PVH using the anterograde autoradiographic method (Saper *et al.*, 1976; Conrad and Pfaff, 1976; Berk and Finkelstein, 1982; Holstege, 1987). Holstege (1987) observed that the medial hypothalamic area, including the PVH, sent fibers to the medulla and spinal cord. The hypothalamic descending fibers were divided into medial and lateral streams, which innervated the vagal nuclear complex and spinal cord. These autoradiographic tracing studies, however, were not able to adequately delineate the route of the descending fibers and projection areas, because the injection sites of tritiated leucine were generally too large and labeling of fibers with silver grains was not appropriate to determine the precise pathways.

Luiten *et al.* (1985) reported the routes of descending projections of the PVH using the PHA-L anterograde tracing method. They indicated two major descending bundles. One fiber tract ran through the mesencephalic periaqueductal grey matter, turned laterally and entered the pedunclopontine tegmental nucleus and the lateral lemniscus. Some fibers projected to the parabrachial nucleus and the nucleus of the locus ceruleus through the central grey matter.

The other fiber bundle coursed through the medial forebrain bundle and the ventral tegmentum. Some fibers turned laterodorsally to the lateral lemniscus and projected to the reticular formation. At the pontine level, these two fiber tracts joined each other at the lateral lemniscus and descended through the medulla oblongata. After projecting to the vagal nuclear complex, the fibers descended through the dorsolateral funiculus and gave collateral projections to the intermediolateral cell column and lamina X through the cervical to the lumbar spinal cord.

Our results were different from those described by Luiten *et al.* (1985). First, the fibers running through the periaqueductal grey matter did not turn ventrally, nor did they join the lateral lemniscus. They generally terminated in the nucleus of the locus ceruleus and the parabrachial nucleus. Some fibers were present in the pontine reticular formation and projected to the pedunclopontine tegmental nucleus, but never traversed it. These discrepancies may have arisen when fibers from the periaqueductal grey matter were confused with those from the ventral tegmentum at the pedunclopontine tegmental nucleus. We believe that the main fiber tracts projecting to the spinal cord course through the medial forebrain bundle, the ventral tegmentum along the interpeduncular nucleus, and the ventrolateral part of the pontine tegmentum to the medulla oblongata.

A second discrepancy involves their description of all spinal projection fibers running through the dorsolateral funiculus. We found that the fibers after projecting to the vagal nuclear complex turned caudolaterally and entered lamina V (Figs 3, E, F), then gave terminal-like boutons to lamina I at all levels of the spinal cord (Fig. 5, A, B). Some of the fibers projected to the spinal trigeminal nucleus. Even at the coccygeal spinal cord, there were labeled terminal-like boutons in lamina I (Fig. 6, C). There were no fibers coming from the lateral funiculus to lamina I. This is an important finding of our study. It was made possible by use of a long survival time of the PHA-L tracer. These results suggest that PVH neurons regulate nociceptive and somatosensory inputs to all parts of the body (Brown, 1981). Fibers running in the lateral reticular nucleus joined the dorsolateral funiculus, and gave terminal-like boutons to all regions of the intermediolateral cell column and sacral parasympathetic nucleus. This agrees with studies by Holstege (1987) and Schwanzel-Fukuda *et al.* (1984). In the cat there were some labeled neurons in Onuf's nucleus (Holstege, 1987). Although we observed only a few labeled fibers, there were no terminal-like boutons in the ventral horn of the sacral spinal cord. As Onuf's nucleus is not prominent in the rat, few fibers of the PVH project to this nucleus.

The interesting aspect of this study is that lamina X received many terminal-like boutons from C5 to Sc. Neurons of lamina X contain noxious specific cells as well as sympathetic preganglionic neurons (Nahin *et al.*, 1983; Ugolini, 1992). Thus the PVH neurons in the dorsolateral funiculus may regulate sympathetic, parasympathetic as well as nociceptive inputs in this region (De Groat *et al.*, 1981; Nahin *et al.*, 1983; Ugolini, 1992). There were also labeled fibers in the lateral funiculus at the coccygeal spinal cord, but no labeled terminal-like boutons there. Swanson and Mckellar (1979) showed oxytocin containing fibers and terminal-like boutons in the coccygeal spinal cord and the filum terminale. Then these labeled fibers may terminate at the filum terminale.

The PVH neurons contain many kinds of neurotransmitters, such as oxytocin, vasopressin, somatostatin, dopamine, enkephalin, neurotensin, cholecystokinin, dynorphin, substance P and corticotropin releasing factor (see Swanson and Sawchenko, 1983 for a review). Double labeling studies of retrograde tracing and immunohistochemistry revealed that a few neurons of the PVH projecting to the parabrachial nucleus contain enkephalin (2%), dynorphin (1%), corticotropin releasing factor (CRF, less than 1%), galanin (less than 1%) and neurotensin (less than 1%). A few oxytocin containing neurons project to the parabrachial nucleus (Moga *et al.*, 1990). Neurons projecting to the thoracic spinal cord contain many vasopressin (35%), oxytocin (25%) and enkephalin (10%) (Cechetto and Saper, 1988). About one-third of the neurons projecting to the vagal nuclear complex or the thoracic spinal cord contain vasopressin and corticotropin releasing factor (Sawchenko, 1987).

Immunohistochemical studies have described the descending pathway of neurophysin or oxytocin containing fibers (Swanson and Mckellar 1979). These immunopositive fibers distributed to the dorsomedial medulla including the vagal nuclear complex, the lateral reticular nucleus, the dorsolateral funiculus at the cervical to the coccygeal spinal cord, the intermediolateral cell column, the sacral parasympathetic nucleus, lamina X and lamina I. Thus, it may be concluded that the PVH neurons project to three different regions. Neurons with oxytocin and vasopressin project to the medullary parasympathetic and spinal nociceptive neurons including the vagal nuclear complex and lamina I. Neurons with oxytocin and vasopressin project to the sympathetic and parasympathetic preganglionic spinal neurons including the intermediolateral cell column, the sacral parasympathetic nucleus and lamina X. Neurons with little oxytocin and vasopressin project to the mid-brain limbic or extra pyramidal motor neurons in-

cluding the pedunculopontine tegmental nucleus, the nucleus of the locus ceruleus and the parabrachial nucleus. Although the present study showed that the PVH descending fibers coursed three different pathways, it is unclear whether a single neuron of the PVH sent collateral fibers to these three regions. Using retrograde double labeling of two kinds of fluorescent dyes, Swanson and Kuypers (1980) reported that only 10% of the PVH neurons project to both the vagal nuclear complex and the spinal cord. Thus, the PVH may contain three kinds of neurons projecting to these three different regions.

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Abbreviations for Figures

5SPN	spinal trigeminal nucleus
7G	genu of the facial nerve
7N	facial nucleus
A5	A5 noradrenergic cell group
AHP	anterior hypothalamic area
Amb	ambiguus nucleus
AP	area postrema
Ar	arcuate hypothalamic nucleus
C1, C5, C8	1st, 5th and 8th segments of cervical spinal cord
CC	central canal
CGM	midbrain central gray matter
Co1	upper segment of coccygeal spinal cord
Co2	lower segment of coccygeal spinal cord
DMX	dorsal motor nucleus of the vagus
dp	dorsal parvocellular part of PVH
DRN	dorsal raphe nucleus
DTg	dorsal tegmental nucleus
E-W	Edinger-Westphal nucleus
f	fornix
IC	inferior colliculus
ic	internal capsule

IF	interfascicular nucleus
IML	intermediolateral cell column
IP	interpeduncular nucleus
L2, L6	2nd and 6th segments of lumbar spinal cord
LC	nucleus of the locus ceruleus
Lfu	lateral funiculus
LH	lateral hypothalamic nucleus
LL	nucleus of the lateral lemniscus
Ip	lateral parvocellular region of PVH
LPGi	lateral paragigantocellular nucleus
LRN	lateral reticular nucleus
ml	medial lemniscus
mlf	medial longitudinal fasciculus
mp	medial parvocellular part of PVH
MRN	median raphe nucleus
mt	mammillothalamic tract
PBN	parabrachial nucleus
pm	posterior magnocellular part of PVH
PPT	pedunculo pontine tegmental nucleus
pv	periventricular part of PVH
PV	paraventricular thalamic nucleus
PVH	paraventricular hypothalamic nucleus
Re	reuniens thalamic nucleus
RLi	rostral linear nucleus of the raphe
RMg	raphe magnus nucleus
RPa	raphe pallidus nucleus
I, IV, V, VI, VII, X	Rexed's laminae I, IV, V, VI, VII and X of spinal cord
Sa, Sb, Sc	segments a, b and c of sacral spinal cord
scp	superior cerebellar peduncle
SNR	substantia nigra, pars reticularis
Sol	nucleus of the solitary tract
SO	superior olivary nucleus
SPN	sacral parasympathetic nucleus
T3, T8	3rd and 8th segments of thoracic spinal cord
V3	3rd ventricle
VMH	ventromedial hypothalamic nucleus

Explanation of Figures

Plate I

Figs. 1A and B. Schematic representation of PHA-L injection sites (shaded area) in the middle (A) and caudal (B) region of the paraventricular hypothalamic nucleus. C. Light photomicrograph of the PHA-L injection site in the middle part of the paraventricular hypothalamic nucleus in Case P35. Bar is 100 μ m in C.

Plate II

Figs. 2 A-Co2. Schematic representation of the distribution of PHA-L labeled fibers (dashed lines) and terminal-like boutons (dots) in the brainstem and spinal cord after injection into the middle part of the paraventricular hypothalamic nucleus (Case P35).

Plate III

Fig. 3. Photomicrograph of PHA-L labeled fibers and terminal-like boutons. A. Labeled terminal-like boutons in the ambiguous nucleus. Note PHA-L labeled fibers curving dorsomedially (arrows) to the vagal nuclear complex. B. PHA-L labeled fibers and terminal-like boutons in the lateral reticular nucleus. C. PHA-L labeled fibers and terminal-like boutons in the marginal area of the spinal trigeminal nucleus. D. Dense PHA-L labeled fibers and terminal-like boutons in the bilateral nucleus of the solitary tract and the dorsal motor nucleus of the vagus. Note a few labeled fibers in the area postrema. E. The PHA-L labeled fibers enter the ventrolateral region (arrow) and exit from the lateral part of the vagal nuclear complex (double arrow). F. PHA-L labeled fibers in lamina V (V) of the uppermost segment of the cervical cord. Double arrow indicates fibers running from the vagal nuclear complex (arrow) to lamina V. Bar is 100 μ m in A, B, C, E and F, 300 μ m in D.

Plate IV

Fig. 4 A. Photomicrograph of PHA-L labeled fibers and terminal-like boutons in lamina I of C5. B. PHA-L labeled fibers and terminal-like boutons in lamina X of L2. C. Low power photomicrograph of the intermediolateral cell column (IML) of T8. Double arrow indicates labeled fibers running through the IML to lamina X after giving terminal-like boutons in the IML. D. High power photomicrograph of labeled fibers and terminal-like boutons in the IML of T8. Counterstained with thionin. Bar is 50 μ m in A and B, 100 μ m in C, and 20 μ m in D.

Plate V

Fig. 5A and B. Photomicrographs of two bundles of PHA-L labeled fibers running through lamina I (arrowheads) and the dorsal part of lateral funiculus (open arrows) at the level of T12 (A) and L4 (B) of spinal cord. Arrow indicates labeled fibers running from lamina V to lamina I. Bar is 100 μ m in A and 300 μ m in B.

Plate VI

Fig. 6 A. Low power photomicrograph of PHA-L labeled fibers and terminal-like boutons in the a-segment of the sacral cord. Double arrow indicates labeled fibers running from the dorsal part of the lateral funiculus (Lfu) to lamina X (X) after giving terminal-like boutons in the sacral parasympathetic nucleus (SPN). B. High power photomicrograph of PHA-L labeled fibers and terminal-like boutons in the sacral parasympathetic nucleus of the a-segment of the sacral cord. Counterstained with thionin. C. Photomicrograph showing PHA-L labeled fibers in the lateral funiculus (Lfu) and terminal-like boutons in lamina I (arrows) of the coccygeal spinal cord. Bar is 100 μm in A and C, 20 μm in B.

Plate VII

Fig. 7 Schematic representation of the main descending pathways from the paraventricular hypothalamic nucleus to the spinal cord based on our investigation. Note the descending projections to the spinal cord divided into two pathways at the level of the lateral reticular nucleus.