The afferent connections of the posterior hypothalamic nucleus in the rat using horseradish peroxidase

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ABSTRACT

The posterior hypothalamic nucleus has been implicated as an area controlling autonomic activity. The afferent input to the nucleus will provide evidence as to its role in autonomic function. In the present study, we aimed to identify the detailed anatomical projections to the posterior hypothalamic nucleus from cortical, subcortical and brainstem structures, using the horseradish peroxidase (HRP) retrograde axonal transport technique in the rat. Subsequent to the injection of HRP into the posterior hypothalamic nucleus, extensive cell labelling was observed bilaterally in various areas of the cerebral cortex including the cingulate, frontal, parietal and insular cortices. At subcortical levels, labelled cells were observed in the medial and lateral septal nuclei, the bed nucleus of stria terminalis, and various thalamic and amygdaloid nuclei. Also axons of the vertical and horizontal limbs of the diagonal band were labelled and labelled cells were localised at the CA1 and CA3 fields of the hippocampus and the dentate gyrus. The brainstem projections were from the medial, lateral and parasolitary nuclei, the intercalated nucleus of the medulla, the sensory nuclei of the trigeminal nerve, and various reticular, vestibular, raphe and central grey nuclei. The posterior hypothalamic nucleus also received projections from the lateral and medial cerebellar nuclei and from upper cervical spinal levels. The results are discussed in relation to the involvement of the posterior hypothalamic nucleus in autonomic function and allows a better understanding of how the brain controls visceral function.

Key words: Hypothalamus; autonomic function.

INTRODUCTION

The hypothalamus plays an important role in autonomic and behaviour control mechanisms. Of all the hypothalamic nuclei, the posterior nucleus may be considered as anatomically the least clearly understood. Previous studies using lesion and stimulation techniques suggested that this nucleus has a vital role in cardiovascular homeostasis, cardiorespiratory activity and locomotion (Eferakeya & Bunag, 1974; Buccafusco & Brezenoff, 1979; Yardley & Hilton, 1986; Waldrop et al. 1988; Martin, 1992). In addition to its role in cardiac regulation, it may also influence the control mechanisms of many organs through its neural connections.

A number of studies have revealed various efferent projections from the posterior hypothalamic nucleus. In an autoradiographic study, caudal hypothalamic projections to the hippocampus have been demonstrated in the cat (Stanfield & Cowan, 1984) and, Nomokonova & Ozirskaya (1984) reported posterior hypothalamic projections to forebrain structures in the pond turtle. However, there is still a conspicuous lack of information as to the afferent input to the posterior hypothalamic nucleus which remains a matter of discussion. Such projections would provide strong evidence as to the functional role of this nucleus within the central nervous system. Using the horseradish peroxidase (HRP) retrograde axonal transport technique we therefore aimed to identify the afferent projections from cortical, subcortical and brainstem regions to the posterior hypothalamic nucleus in the rat.
MATERIALS AND METHODS

Animals

Sprague–Dawley rats weighing 200–250 g were allowed a standard laboratory rat chow and tap water ad libitum and housed in Plexiglass cages in a 12 h light/dark cycle and a temperature-controlled room (20 ± 3 °C). All procedures were approved by the Institutional Animal Care and Use Committee of Marmara University.

HRP labelling

The animals were anaesthetised with ketamine (50 mg/kg intraperitoneally; i.p.) and chlorpromazine (1 mg/kg i.p.). The head of the rat was positioned in a stereotaxic apparatus (Stoelting model 51600, USA). A glass micropipette (10–18 µm) filled with 100 nl solution of HRP was lowered into the right posterior hypothalamic nucleus (nfl 7) using stereotaxic coordinates adapted from the rat brain atlas of Paxinos & Watson (1998). The coordinates for the posterior hypothalamic nucleus were 4–1 mm caudal and 0–5 mm lateral to the bregma and 8–0 mm vertical to the surface of the skull (Fig. 1). A volume of 100 nl of HRP was applied by pressure injection via a Hamilton microsyringe through the pipette connected to an infusion pump (Kd Scientific, USA). After injection of HRP, the micropipette remained at the target site for 1.5 h to avoid flushing of HRP during removal of the pipette, and to avoid loss of tracer in the pipette track. After 2–3 d survival, the animals were anaesthetised with ether and perfused transcardially with 500 ml of saline followed by 1 % of paraformaldehyde 1.25 % glutaraldehyde in a 0.05 M phosphate buffer (500 ml). The brains were removed, postfixed for 24 h at 4 °C and sectioned coronally at 30–50 µm using a cryostat (Microm, FRG). Sections were collected in phosphate buffer (pH 7.7) and treated with tetramethylbenzidine as described by Mesulam (1978). The HRP injection sites were examined microscopically to verify the location. Only proper placements were included in the study.

HRP labelled neuron counts

HRP labelled neurons were counted at ×400 magnification. An eyepiece graticule (covering 0.07850 mm²) was used to define the counting area. At least 15 such area in each region were surveyed for HRP-labelled neurons; density was then expressed as cell numbers per unit area. The total estimated cell numbers were determined by multiplying by 10.

RESULTS

The posterior hypothalamic nucleus was localised on the medial wall of the hypothalamus and extended between the mamillothalamic tract and the wall of the third ventricle. Anteriorly it was bounded by the posterior hypothalamic area and the dorsomedial

Fig. 1. Photomicrograph combined with a schematic drawing of a coronal section of the brain showing the injection site (→) into the posterior hypothalamic nucleus (Bregma −4.16).
hypothalamic nucleus, posteriorly by the mamillary nuclei (Fig. 1).

Subsequent to the placement of HRP into the posterior hypothalamic nucleus, a central core surrounded by a diffuse spread of tracer was observed. The central core was considered to be the effective site for transport of the HRP.

Cortical projections to the posterior hypothalamic nucleus

At 2–3 d following microinjection of HRP into the posterior hypothalamic nucleus, labelled cells were observed in various regions of the cerebral cortex. On the ipsilateral side, numerous densely labelled cells closely encircled the forceps minor of the corpus callosum (Cg1, Cg3, Fr1, Fr2, Fr3, Al, LO, VLO, MO, VO; for explanation of these and subsequent abbreviations, see Appendix), whereas on the contralateral side, fewer cells were lightly labelled at similar regions. No labelled cells were observed in the frontal cortex. It was striking that the labelled cells in the cingulate cortex contralaterally were present at regions 1.70 mm anterior to the bregma; labelled cells at the cingulate cortex gradually disappeared posterior to this level. Further, labelled cells were consistently observed in the insular cortex bilaterally (GI, AI, DI) with an ipsilateral predominance. The labelled cells in the ipsilateral cingulate and frontal cortices were scattered through the 6 layers and showed no regular labelling whereas, in the parietal and insular cortices, labelled cells were localised in distinct cortical layers, specifically the external pyramidal (cortical layer III) and the internal pyramidal (cortical layer V) (Fig. 2). The labelled cells in the external pyramidal layer were mainly large size pyramidal cells, whereas labelled cells in the internal pyramidal layer were small and medium size pyramidal cells. The number of labelled cells in the internal and external pyramidal layers in the various cortical areas were counted per/unit area and are given in Figure 3. Additionally, moderate to light retrogradely labelled cells were observed ipsilaterally in the entorhinal, perirhinal, occipital (Oc1,
Fig. 4. Bar graph showing the number of labelled per unit area cells in specific regions in subcortical and brainstem structures following HRP injections into posterior hypothalamic nucleus. Solid columns, ipsilateral; stippled columns, contralateral.

Fig. 5. Photomicrograph showing HRP-positive cells of the spinal nucleus of the trigeminal nerve (Sp5). × 600.

Oc2), dorsal peduncular, infralimbic and orbital (lateral and ventrolateral) cortices.

**Subcortical projections to the posterior hypothalamic nucleus**

The retrograde labelling of subcortical structures was predominantly ipsilateral although there was moderate to light labelling on the contralateral side. At the level of the decussation of the anterior commissure, predominant ipsilateral labelling was present in the medial and lateral (LSD, LSV and LSI) septal nuclei localised on the medial border of the lateral ventricle (Fig. 4). Further caudally moderately labelled cells were observed in the bed nucleus of stria terminalis (BSTMA and BSTV) and ventral pallidum (Fig. 4). Additionally, at this level the axons of the vertical and
horizontal limb of the diagonal band were labelled bilaterally. Further, moderately labelled cells were observed in the lateral (LaDL, LaVM and LaVL), basolateral (BLA, BLP, BMP), medial and postero-medial cortical amygdaloid nuclei (Fig. 4). Labelled cells were also observed in the amygdalohippocampal area, the CA1 and CA3 fields of the hippocampus, the dentate gyrus, subiculum and presubiculum. At thalamic levels, ipsilateral labelled cells were localised in the lateral posterior thalamic nucleus, intramedullary area, dorsolateral geniculate nucleus, intergeniculate leaf and medial geniculate nucleus.

**Brainstem projections to the posterior hypothalamic nucleus**

Following HRP injections into the posterior hypothalamic nucleus, the majority of projections from the brainstem were bilateral. Dense bilaterally labelled cells were observed in the medial solitary, lateral solitary and parasolitary nuclei (Fig. 4). At medullary levels, densely labelled cells were observed in both intercalated nuclei of the medulla located on each side of the fourth ventricle. Further cranially densely labelled axons were observed in the predorsal bundle.
on each side of the midline of the medulla. Distinctly labelled cells were seen in all 3 trigeminal sensory nuclei: (greatest to least concentration) spinal (Sp5, SpVe) (Fig. 5), principal and mesencephalic. Moderately labelled cells were seen in various reticular nuclei of the brainstem such as the lateral, medullary, rostroventral-lateral (Fig. 6) and intermediate reticular, and the gigantocellular and lateral paragigantocellular nuclei.

At mesencephalic levels lightly labelled cells were observed ipsilaterally in tegmental nuclei including the pedunculopontine tegmental, reticulotegmental and lateral dorsal tegmental nuclei. At pontine levels, densely labelled cells were observed in the medial and lateral vestibular nuclei (Figs 4, 7). Occasional labelled cells were observed in the superior paraolivary and medial superior olivary nuclei. Additionally, densely labelled cells were seen in various raphe nuclei, including the dorsal raphe, raphe pontine, median raphe, and paramedian raphe nuclei. Faintly labelled cells were also observed in the dorsal nucleus of the lateral lemniscus. Additionally, a few cells were labelled in the dorsal and external cortex of the inferior colliculus, and the superficial layer of the superior colliculus. Further, labelled cells were observed in the lateral parabrachial nucleus and locus coeruleus, anterior pretectal nucleus and pars compacta of the substantia nigra (Figs 4, 8).
Cerebellar projections to the posterior hypothalamic nucleus

The posterior hypothalamic nucleus also received dense bilateral projections from the cerebellum, predominantly from the ipsilateral side. Numerous cells of the lateral (Lat, LatPC) and medial cerebellar nuclei were labelled with HRP (Fig. 9). The HRP labelled cells of the contralateral lateral cerebellar nuclei showed faint labelling and lower cell counts (Fig. 4). The HRP labelled axons were transmitted via the superior cerebellar peduncle.

Spinal cord projections to the posterior hypothalamic nucleus

At upper cervical spinal cord levels, labelled cells were observed at the medial border of the anterior horn. A few labelled cells were seen at the base of the posterior horn. No labelled cells were detected at any other spinal level.

DISCUSSION

The results of our study have shown that the posterior hypothalamic nucleus receives afferents from various cortical, subcortical and brainstem structures which are involved in autonomic regulation. These include the insular cortex, septal nuclei, amygdala, subiculum, bed nucleus of stria terminalis, central grey, parabrachial nucleus, nucleus of the solitary tract and brainstem reticular nuclei.

The study has also shown that the posterior hypothalamic nucleus receives extensive projections from various cortical regions such as the cingulate, frontal, parietal and insular cortices. Connections from the prefrontal and occipital cortices to the posterior hypothalamus have been shown in the cat (Kitahama et al. 1984; Wouterlood et al. 1987) Our study confirms both the prefrontal and occipital cortical connections to the posterior hypothalamus in the rat. The somatomotor and medial prefrontal cortical regions have been considered likely autonomic control sites in the cortex and have been implicated in integrating behavioural and autonomic responses (Hoff et al. 1963; Cechetto & Chen, 1990; Allen et al. 1991). Further, studies have shown that the insular cortex receives a visceral input and interconnects with the autonomic nuclei of the forebrain (Cechetto & Saper, 1987; Hörster & Ettlinger, 1987; Yasul et al. 1991). The present study clearly demonstrated constant bilateral connections of the insular cortex and other cortical areas with the posterior hypothalamic nucleus, suggesting the participation of these areas in the integration of autonomic responses.

The lateral and medial septal nuclei are forebrain structures which are part of the limbic system. Connections of the septal nuclei have been demonstrated with the lateral, dorsomedial and ventromedial hypothalamic nuclei (Swanson & Cowan, 1979). The present study also showed connections of the septal nuclei with the posterior hypothalamic nucleus. The amygdala is another autonomic centre which has been known to be an important component of the central pathways that mediate autonomic and somatomotor responses to emotional stimuli in a variety of species (Blanchard & Blanchard, 1972; Cohen, 1975). Electrical stimulation of the amygdala in the conscious cat elicits cardiovascular changes such as an increase in blood pressure (Timms, 1981). We have described distinct projections from lateral, basolateral, medial and posteromedial amygdaloid nuclei to the posterior hypothalamic nucleus. Anatomical studies have shown that the amygdala receives both direct and indirect input from the solitary tract which is the principal site of termination of primary afferent fibres arising from cardiovascular and many other visceral and somatic receptors (Saper & Loewy, 1980). These results together with the results of the present study may suggest that amygdala-posterior hypothalamic connections may play an important role in interconnecting autonomic inputs from the amygdala to the posterior hypothalamic nucleus.

The evidence related to the parabrachial nucleus both anatomically and functionally clearly indicates that the parabrachial nucleus is an important component of the pathway relaying visceral afferent (mainly from the solitary tract) information to forebrain nuclei (Herbert et al. 1990). The present study confirms anatomically the connection of the parabrachial nucleus to the posterior hypothalamic nucleus.

The midbrain periaqueductal grey is another centre which is involved in autonomic circuity and has a direct connection with the posterior hypothalamic nucleus. The periaqueductal grey has been known to play an important role in functions such as antinociception and defensive behaviour (Basbaum & Fields, 1984). Further, recent anatomical and physiological studies have shown its role in cardiovascular control (Tan & Dampney, 1983). The posterior hypothalamus along with many other forebrain areas has a function in regulating the periaqueductal grey with regard to its role as a visceral nociceptive and cognitive integrator (Mantyh, 1982). Injections of tracer into the midbrain
periaqueductal grey showed labelled neurons in the posterior hypothalamus (Mantyh, 1982). The result of the present study and that by Mantyh (1982) demonstrated a reciprocal connection between the periaqueductal grey and the posterior hypothalamic nucleus.

The rostroventrolateral reticular nucleus (RVL) of the medulla has been regarded as a sympathetic premotor centre that regulates the preganglionic outflow to major sympathetic ganglia (Strack et al. 1988). Excitation of the RVL cells by local micro-injection of excitatory amino acids produces an increase in blood pressure, heart rate and the release of adrenomedullary catecholamines (Strack et al. 1988; McAllen & Dampney, 1989). McAllen & Dampney (1990) demonstrated projections from the RVL to the lateral and posterior hypothalamus which was confirmed in the present study.

The raphe nuclei are one of the major sources for the descending input to the intermediolateral cell column, the axons of which synapse with sympathetic preganglionic neurons that innervate the adrenal medulla and major sympathetic ganglia (Miura et al. 1983). The present study demonstrates a direct connection from the raphe nuclei to the posterior hypothalamic nucleus. These findings suggest that the posterior hypothalamic nucleus may take part in regulating sympathetic activity through the intermediolateral cell column.

The efferent projections of the posterior hypothalamic nucleus have been studied using autoradiography, showing connections with the tegmental field of the midbrain, the raphe complex, the locus coeruleus and the facial nerve (Veaey et al. 1982). The result of the present study showed afferent connections to the posterior hypothalamic nucleus from the former areas of the brain which confirmed reciprocal connections.

Sakai et al. (1990) demonstrated lower brainstem afferents to the cat posterior hypothalamus and observed projections from the substantia nigra, peripeduncular nucleus, ventral tegmental area, periaqueductal grey, mesencephalic reticular formation, locus coeruleus, rostral raphe nuclei and the rostral part of the nucleus magnus. These projections observed in the cat strongly confirm our results in the rat. In addition to these connections, we also observed dominant connections with the solitary, trigeminal and vestibular nuclei and the superior and inferior colliculi. These additional connections may reflect species differences.

Studies with tracing methods in various species have revealed hypothalamocerebellar and cerebello-hypothalamic projections (Dietrichs, 1984; Dietrichs & Haines, 1984; Haines et al. 1984, 1985, 1990; Çavdar et al. 2000). In this study, we have revealed distinct connections to the posterior hypothalamic nucleus from the lateral (dentate) and medial (fastigial) cerebellar nuclei. Stimulation and/or ablation of the cerebellar cortex or nuclei may elicit or modify a wide range of visceral responses, including piloerection, changes in blood pressure, heart rate and respiration, alterations in smooth muscle tone (bladder, pupil, intestines, nictitating membrane), urination and increased cerebral blood flow (Zheng et al. 1982; Shapiro & Miselis, 1985; Chida et al. 1986). The findings of this study suggest a new perspective on the question of how the cerebellum may influence autonomic activity.

The present anatomical data gain further understanding of the wide neural connections of the posterior hypothalamic nucleus with distinct direct afferent connections with various nuclei involved in autonomic functions at cortical, subcortical and brainstem levels and how the posterior hypothalamic nucleus may influence autonomic activity.

ACKNOWLEDGEMENTS

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Al</td>
<td>agranular insular cortex</td>
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<tr>
<td>BLA</td>
<td>basolateral amygdaloid nucleus, anterior part</td>
</tr>
<tr>
<td>BLP</td>
<td>basolateral amygdaloid nucleus, posterior part</td>
</tr>
<tr>
<td>BMP</td>
<td>basomedial amygdaloid nucleus, posterior part</td>
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<tr>
<td>BSTMA</td>
<td>bed nucleus of stria terminalis, medial division, anterior part</td>
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<tr>
<td>BSTV</td>
<td>bed nucleus of stria terminalis, ventral division</td>
</tr>
<tr>
<td>CA1, CA3</td>
<td>Ammon’s horn</td>
</tr>
<tr>
<td>Cg1, Cg2, Cg3</td>
<td>cingulate cortex, area 1,2</td>
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<tr>
<td>DI</td>
<td>dysgranular insular cortex</td>
</tr>
<tr>
<td>DR</td>
<td>dorsal raphe nucleus</td>
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<tr>
<td>Fr1, Fr2 Fr3</td>
<td>frontal cortex, area 1,2,3</td>
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<tr>
<td>GI</td>
<td>granular insular cortex</td>
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<td>LaDL</td>
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<td>Lat</td>
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<td>LO</td>
<td>lateral orbital cortex</td>
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