Cerebellar connections to the dorsomedial and posterior nuclei of the hypothalamus in the rat

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(*Accepted 3 July 2000*)

ABSTRACT

The stimulation or ablation of cerebellar structures has produced a variety of visceral responses, indicating a cerebellar role in visceral functions. Studies using anterograde and retrograde tracing methods have revealed connections between the hypothalamus and cerebellar structures. The aim of this study is to investigate the cerebellar connections of the dorsomedial (DMH) and posterior hypothalamic nuclei using retrograde axonal transport of horseradish peroxidase (HRP). In the present study, micro-injection of HRP restricted within the borders of the DMH showed that the projections of this nucleus are not uniform throughout its extent. The posterior DMH receives projections from the cerebellum, whereas the anterior DMH does not. These projections were from the (greatest to least concentration) lateral (dentate), anterior interposed (emboliform), and medial (fastigial) cerebellar nuclei. In addition, both the anterior and posterior DMH receive projections from various areas of the brainstem which confirms earlier studies and provides detailed descriptions. This study also demonstrates the distribution of labelled neurons to cerebellar and brainstem nuclei following HRP injection into the posterior hypothalamic nucleus. It provides clear evidence for a direct cerebellar nuclei-posterior DMH and cerebellar nuclei-posterior hypothalamic nucleus connections. We suggest that the brainstem reticular nuclei and other connections, such as the solitary, trigeminal and vestibular nuclei, of both DMH and posterior hypothalamus may contribute to the indirect cerebellohypothalamic connections. These observations offer a new perspective on the question of how the cerebellum may influence autonomic activity.

Key words: Dorsomedial hypothalamic nucleus; posterior hypothalamic nucleus; cerebellum.

INTRODUCTION

The cerebellum controls the coordination of somatic motor activity, the regulation of muscle tone and mechanisms that influence and maintain equilibrium. The role of the cerebellum in the regulation of somatic motor activity has been well established in various species (Bloedel, 1973; Anderson et al. 1987). Some early investigations offered evidence for the involvement of the cerebellum in visceral functions. Early evidence for cerebellohypothalamic connections was presented by Wallenberg (1905). He described degenerating axons in the rabbit hypothalamus after a brainstem lesion which included the brachium conjunctivum. Further, Whiteside & Shider (1953) recorded short-latency evoked potentials from cerebellar cortex following hypothalamic stimulation, and from the hypothalamus subsequent to cerebellar cortical stimulation. Jacobs (1965) reported degeneration in the hypothalamus following a lesion of the superior cerebellar peduncle and the lateral and anterior interposed cerebellar nuclei. Martin et al. (1974) described degeneration in the lateral hypothalamic area following destruction of the medial cerebellar nucleus. In addition, stimulation or ablation of the cerebellar cortex or nuclei may elicit or modify a wide range of visceral responses [piloerection, changes in blood pressure, heart rate and respiration, alterations in smooth muscle tone (bladder, pupil, intestines, nictitating membrane), micturation and an

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increase in cerebral blood flow] (Zheng et al. 1982; Chida et al. 1986). Likewise, the dorsomedial hypothalamic nucleus (DMH) and the posterior nucleus of the hypothalamus have been implicated in a variety of behavioural and physiological responses, including those associated with ingestion, reproduction and endocrine and autonomic functions (Dalton et al. 1981; Waldrop et al. 1988; Inglefield et al. 1994). Recent studies demonstrated involvement of the DMH in the central control of cardiovascular regulation. Activation of $GABA_A$ or N-methyl-*D*-aspartate (NMDA) receptors in the DMH produced tachycardia and pressor responses in both conscious and anaesthetised rats (Gören et al. 1996, 2000). Bilateral destruction of the DMH results in alterations of electrical activity in the pancreatic branch of the vagal and splanchnic nerves (Yoshimatus et al. 1984). On the other hand, electrical stimulation of the posterior hypothalamic nucleus of anaesthetised rats is known to evoke locomotion as well as increased cardiorespiratory activity (Eferakeya & Bunag, 1974). Recent clinical studies have also shown evidence of a cerebellar influence on the visceromotor system in patients with a defect in the medial, anterior and posterior interposed cerebellar nuclei (Haines et al. 1997).

A number of neuroanatomical studies in various species (cat, rat, tree shrew, bushbaby, squirrel monkey and prosimian) using neurophysiological, tract tracing, and neurochemical methods have revealed reciprocally organised direct and indirect cerebellohypothalamic and hypothalamocerebellar pathways (Haines & Dietrichs, 1983; Dietrichs & Haines, 1984; Dietrichs & Zheng, 1984; Dietrichs et al. 1985*a*; Haines et al. 1985, 1986). Studies have shown direct projections from the hypothalamus to the cerebellar cortex and nuclei (Dietrichs, 1984; Haines et al. 1984, 1990; Dietrichs & Haines, 1986). Further, direct cerebellohypothalamic projections originating from the cerebellar nuclei and terminating in various nuclei of the hypothalamus have been demonstrated (Dietrichs & Zheng, 1984; Haines et al. 1984; Dietrichs et al. 1985*a*).

Another pathway responsible for hypothalamic involvement in motor responses or cerebellar involvement in nonsomatic activities involves indirect multisynaptic hypothalamocerebellar projections. The indirect hypothalamocerebellar connections are suggested to be through various brainstem reticular nuclei (Ban, 1964; Dietrichs et al. 1985*b*).

In this study, we investigate direct connections of the cerebellum to the DMH and posterior hypothalamic nucleus in rat using the horseradish peroxidase (HRP) tracing technique. These connections are discussed in relation to cerebellar involvement in autonomic regulation.

MATERIALS AND METHODS

Sixteen Wistar albino rats weighing 250–400 g were fed with a standard laboratory rat chow and tap water ad libitum, and housed in Plexiglass cages with a 12 h light/dark cycle in a temperature-controlled room (20 ± 3 °C). All procedures were approved by the Institutional Animal Care and Use Committee of Marmara University.

Rats were anaesthetised with ketamine (50 mg/kg, intraperitoneally (i.p)) and chlorpromazine (1 mg/kg, i.p.). The heads of the animals were placed in a sterotaxic frame (Stoelting Model 51600, USA). The scalps were incised longitudinally, and the skulls were exposed between lambda and bregma. A small hole was drilled in the skull at a position appropriate for the injection of HRP in the right DMH or posterior hypothalamic nucleus. A glass micropipette (10–18 μm) containing the HRP solution was lowered into the right DMH (3.1 mm caudal to bregma, 0.5 mm lateral to the midline and 7.9 mm ventral to the surface of the skull) or posterior hypothalamic nucleus (4.1 mm caudal to bregma, 0.5 mm lateral to the midline and 8.0 mm ventral to the surface of the skull) using stereotaxic coordinates adapted from the rat brain atlas of Paxinos & Watson (1998). The tip of the micropipette was filled with air (20 nl) to avoid diffusion of HRP to unwanted areas of the brain during the lowering procedure of the pipette to the DMH or posterior hypothalamic nucleus. A volume of 100 nl of HRP was applied by pressure injection via a Hamilton microsyringe through the cannula connected to an infusion pump (Kd Scientific, USA). The injections were performed within 10 s with the aid of the pump. Following the injection, the pipette remained in the target location for 1.5 h to avoid loss of tracer during the removal of the pipette.

After 2–3 d survival, the animals were deeply anaesthetised with ether and perfused transcardially with 500 ml of saline, and 1% of paraformaldehyde and 1.25% glutaraldehyde in 0.05 M phosphate buffer (500 ml). The brains were removed and postfixed for 24 h at 4 °C. Coronal sections (30–50 μm) were cut using a cryostat (Microtom, FRG). Sections were collected in phosphate buffer (pH 7.7). The sections were treated with tetramethylbenzidine as described by Mesulam (1978). The HRP injection sites were stained and examined microscopically to verify the

location. Only proper placements were included in the study.

The HRP injection site consisted of a central core (black zone) which is considered to be the effective site for uptake of the transported tracer. Injections from 2.56 to 3.14 mm caudal and 0.5 mm lateral to the bregma and 7.9 mm ventral to the surface of the skull were classified as anterior DMH (n = 5). Injections from 3.14 to 3.6 mm caudal and 0.5 mm lateral to the bregma and 7.9 mm ventral to the surface of the skull were classified as posterior DMH (n = 6; Figs 1, 2). Coordinates for the posterior hypothalamic nucleus (n = 5) were from 3.8 to 4.3 mm caudal and 0.5 mm lateral to the bregma and 8.0 mm vertical to the surface of the skull (Fig. 3).

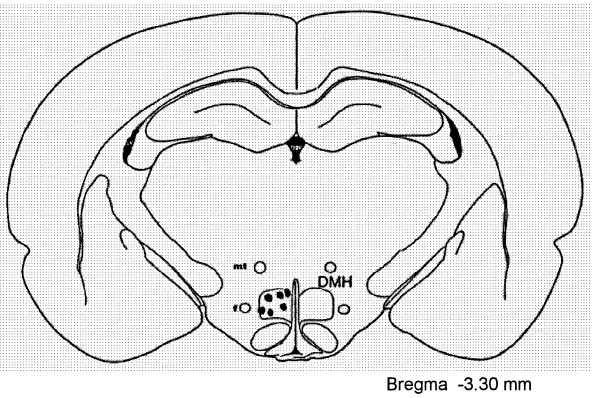


Fig. 2. Schematic drawing of coronal section of the brain showing the injection sites in the posterior DMH (3.3 mm posterior to bregma).

RESULTS

The anteroposterior length of the DMH is ~ 1000 μm and that of the posterior hypothalamic nucleus 900 μm in rats of average weight (~ 250 g). The DMH extends from the bregma −2.56 to −3.60 mm and the posterior hypothalamic nucleus from the bregma −3.6 to −4.5 mm according to the atlas of Paxinos & Watson (1998). Anatomically, the DMH is located on the medial wall of the hypothalamus, dorsal to the ventromedial hypothalamic nucleus and medial to the zona inserta. The anterior hypothalamic area and the paraventricular nucleus comprise the rostral limit. The posterior hypothalamic nucleus and the premamillary nuclei border it caudally. The DMH adjoins the fornix and the prefornical nucleus on the lateral side and medially the periventricular cell layers of the 3rd ventricle (Figs 1, 2). The posterior hypothalamic nucleus extends between the mamillothalamic tract and the wall of the 3rd ventricle.

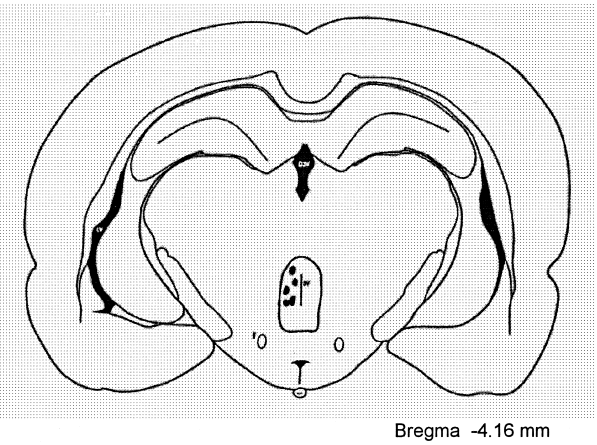


Fig. 3. Schematic drawing of coronal section of the brain showing the injection sites in the posterior hypothalamic nucleus (4.1 mm posterior to bregma).

Anteriorly, the posterior hypothalamic area and the DMH border the posterior hypothalamic nucleus. Posteriorly, it is bordered by the mamillary nucleus (Fig. 3).

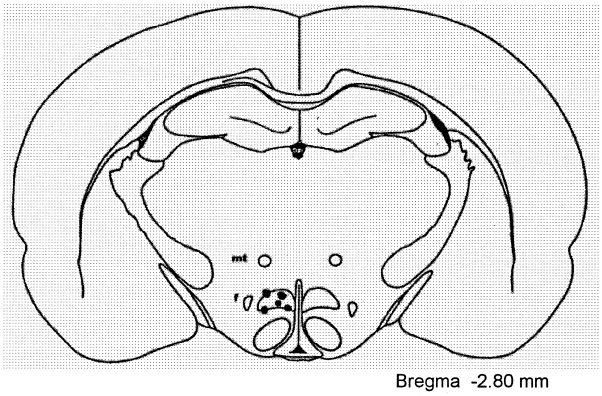


Fig. 1. Schematic drawing of coronal section of the brain showing the injection sites in the anterior DMH (2.8 mm posterior to bregma).

Projections of the dorsomedial hypothalamic nucleus

Our results show major differences in the projections of the cerebellum to the DMH. Subsequent to the placement of tracer in the anterior part of the DMH, significant differences in distribution of the projections were observed compared with the placement of the tracer to the posterior aspect of the DMH. Therefore, the projections of the anterior and posterior DMH were evaluated individually (Tables 1, 2).

It was striking that the anterior DMH received no cerebellar projections, whereas the posterior DMH received distinct bilateral projections from (greatest to

Table 1. *The retrogradely labelled cells at various areas of the brainstem after injections made into the anterior DMH**

	Ipsilateral	Contralateral
LVe	—	○
SpVe	—	○
MVe	—	○
DPGi	○	○
Gi	○	○
SolM	—	○
RVL	○	●
LRt	—	●
7	—	○

* Abbreviations for Tables 1–3. ●, numerous labelled cells with dense HPR granules; ○, sparse labelled cells with faint HPR granules; —, no labelled cells; CG, central grey; DMH, dorsomedial hypothalamic nucleus; DPGi, dorsal gigantocellular nucleus; DR, dorsal raphe nucleus; D3V, dorsal third ventricle; f, fornix; Gi, gigantocellular nucleus; IntA, interposed cerebellar nucleus, anterior part; IRt, intermediate reticular nucleus; Lat, lateral cerebellar nucleus (dentate nucleus); LatPC, lateral cerebellar nucleus, parvocellular part; LRt, lateral reticular nucleus; LV, lateral ventricle; LVe, lateral vestibular nucleus; Med, medial cerebellar nucleus (fastigial nucleus); Me5, mesencephalic nucleus of trigeminal nerve; MnR, median raphe nucleus; MVe, medial vestibular nucleus; mt, mamillothalamic tract; Pd, predorsal bundle; PB, parabrachial nucleus; PCRt, parvocellular reticular nucleus; PMn, paramedian reticular nucleus; PnC, pontine reticular nucleus; RVL, rostroventral reticular nucleus; SpVe, spinal medial vestibular nucleus; Sp5, spinal trigeminal tract; SolL, nucleus solitary tract, lateral; SolM, nucleus solitary tract, medial; Spc, superior cerebellar peduncle; 7, facial nerve nucleus.

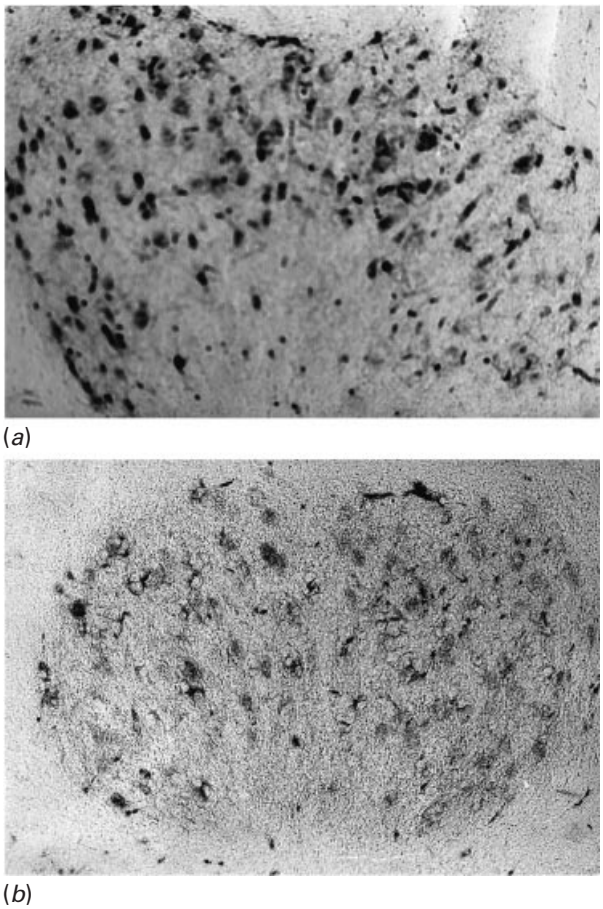


Fig. 4. (*a*) A coronal section of the lateral cerebellar nucleus showing numerous densely HRP labelled cells on the ipsilateral side; (*b*) faintly HRP labelled cells on the contralateral side of the same section, following HRP injections in the posterior DMH.

Table 2. *Retrogradely labelled cells in various areas of the brainstem and cerebellar nuclei after injections into the posterior DMH**

	Ipsilateral	Contralateral
IntA	●	○
Lat	●	○
LatPC	●	○
Med	—	○
MVe	●	○
LVe	○	○
SpVe	—	○
IRt	○	●
DPGi	○	○
Gi	○	○
Sp5	○	●
PCRt	○	○
RVL	○	●
LRt	○	●
PMn	○	○
SolM	—	○
SolL	—	○
PB	—	○
PnC	○	●
CG	○	○

* For explanation of abbreviations see footnote to Table 1.

least concentration) the lateral, the anterior interposed and the medial cerebellar nuclei. The cerebellar projections to the posterior DMH were mainly bilateral, predominantly on the ipsilateral side. The retrogradely labelled cells of the lateral cerebellar nucleus contained varying densities of easily distinguished HRP granules that were similar in shape and size. The HRP labelled cells from the contralateral lateral cerebellar nucleus showed faint labelling compared to the ipsilateral side (Fig. 4*a*, *b*).

Both the anterior and posterior DMH received projections from various areas of the brainstem. The majority of these projections appeared to be from the contralateral side. The anterior DMH received bilateral projections from various brainstem reticular nuclei: rostroventral reticular nucleus (Fig. 5), lateral reticular nucleus, ipsilateral projections from the gigantocellular nucleus and dorsal gigantocellular nucleus. Injections to the anterior DMH showed sparse labelled cells at the medial, lateral, and spinal vestibular nuclei (Table 1). The anterior DMH additionally received sparse contralateral fibres from

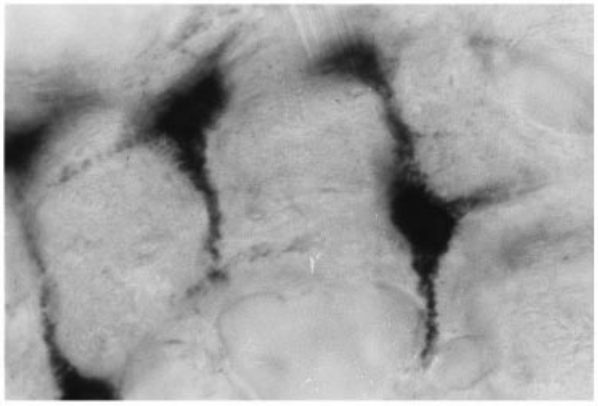


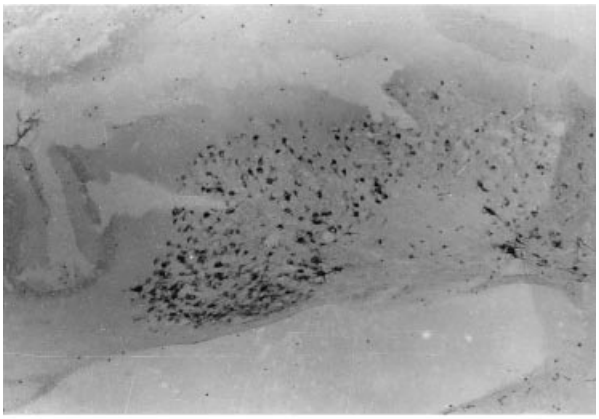
Fig. 5. Photomicrograph showing HRP labelled cells in the rostroventral reticular nucleus of the brainstem, following HRP injections to the anterior DMH.

the facial nerve nucleus and from the solitary nucleus in the medulla.

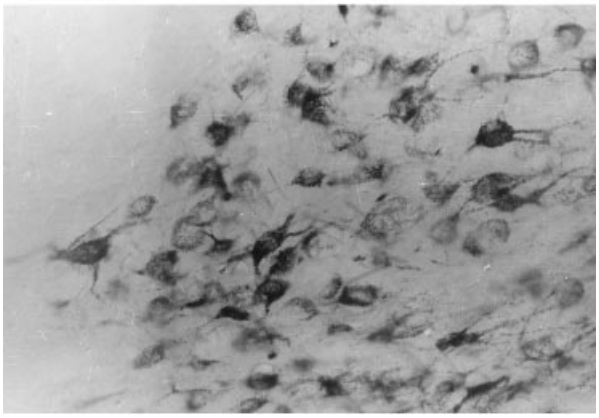
Injections to the posterior DMH showed HRP positive cells in various areas of the brainstem. The posterior DMH received extensive projections from reticular nuclei located in the brainstem. These projections were from rostroventral reticular, paramedian reticular, pontine reticular, lateral reticular, gigantocellular, dorsal gigantocellular, parvocellular reticular and intermediate reticular nuclei and central grey. The majority of these labelled cells were bilateral, being far more extensive and dense on the contralateral side. In addition to the extensive brainstem reticular projections, the posterior DMH also received bilateral projections from the medial, lateral and spinal vestibular nuclei. Further, prominent contralateral projections originated from both medial and lateral solitary nuclei. Distinct bilateral projections from the spinal trigeminal nucleus, parabrachial nucleus were observed (Table 2).

Projections of the posterior hypothalamic nucleus

With respect to its brainstem and cerebellar projections, the posterior hypothalamic nucleus resembles the posterior DMH rather than anterior DMH. Nevertheless, regional differences in the density of various projections were observed. Similar to the posterior DMH, the posterior hypothalamic nucleus also received dense bilateral projections from the lateral and medial cerebellar nuclei, predominantly on the contralateral side (Fig. 6*a*, *b*). The posterior hypothalamic nucleus received extensive projections from the lateral cerebellar nucleus but lesser projections from the medial cerebellar nucleus. No



(*a*)



(*b*)

Fig. 6. (*a*) Photomicrograph showing densely labelled cells in the lateral cerebellar nucleus following HRP injections into the posterior hypothalamic nucleus; (*b*) higher magnification photomicrograph of *a*.

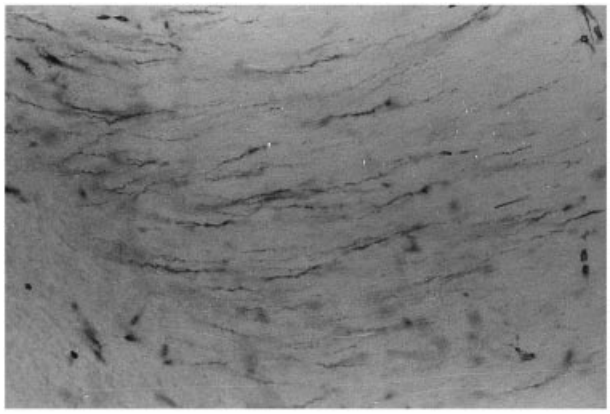


Fig. 7. Photomicrograph showing HRP labelled axons in the superior cerebellar peduncle.

labelled cells were observed in the cerebellar cortex. Furthermore, HRP labelled axons were observed in the superior cerebellar peduncles (Fig. 7).

In addition to these observations, the posterior hypothalamic nucleus received distinct projections

Table 3. *Retrogradely labelled cells in various areas of the brainstem and cerebellar nuclei after injections into the posterior hypothalamic nucleus**

	Ipsilateral	Contralateral
Lat	○	●
LatPC	—	●
Med	○	○
LVe	●	○
MVe	●	○
SpVe	●	○
pd	●	●
IRt	—	○
Sp5	—	○
Me5	—	○
SolM	—	○
SolL	—	○
Gi	○	○
LRt	○	○
RVL	○	○
PMn	○	○
scp	○	○
DR	○	○
PB	—	○
CG	○	○

* For explanation of abbreviations see footnote to Table 1.

from various areas of the brainstem. These projections were from both lateral and medial solitary nuclei, as well as the spinal and mesencephalic nuclei of the trigeminal nerve, the lateral medial and spinal vestibular nuclei and olivary nucleus. We observed extensive projections from various brainstem reticular nuclei, including rostroventral reticular, lateral reticular, intermediate reticular, gigantocellular, paramedian reticular, dorsal raphe, median raphe, lateral paragigantocellular and parabrachial nuclei, central grey and predorsal bundle which were mainly bilateral (Table 3).

DISCUSSION

Our study has shown that the DMH nucleus does not have uniform projections throughout its extent. Injections in the anterior DMH show different projections compared with the posterior DMH. The anterior DMH has no cerebellar connections, whereas these are distinct for the posterior DMH. Ter Horst & Luiten (1986) studied the projections of the DMH using Phaseolus vulgaris leuco-agglutinin immunocytochemical method. They also observed cerebellar deposits only after injections in the area dorsal to the pars compacta but did not define the different connections of the anterior and posterior DMH.

Neuroanatomical studies have shown that the cerebellum and hypothalamus are interconnected by direct and indirect pathways. The presence of direct hypothalamocerebellar and cerebellohypothalamic projections has been described in various species in numerous neuroanatomical studies by Haines and his co-workers (Dietrichs & Zheng, 1984; Haines et al. 1984, 1985, 1986, 1990; Dietrichs et al. 1985*a*). Haines et al. (1985) injected WGA-HRP complex to the cerebellar cortex of a tree shrew and observed retrogradely labelled cells in the posterior, lateral and dorsal hypothalamic areas, and in the lateral mamillary nucleus. Haines et al. (1990) showed that subsequent to a placement of tracer in the posterior interposed cerebellar nuclei, retrogradely labelled cells were found in the ventromedial, dorsomedial, lateral hypothalamic area, perifornical nucleus and periventricular area. They concluded that the DMH, dorsal and lateral hypothalamic areas were the primary sources of hypothalamic input to the cerebellar nuclei. These hypothalamic areas not only make the greatest contributions to the cerebellar projections but also receive direct cerebellar nuclear projections. Moreover, injections into the medial cerebellar nucleus resulted in labelled cells at the dorsal region of the lateral hypothalamic area, dorsal hypothalamic area and in the DMH and posterior hypothalamic nucleus (Haines et al. 1990).

Our specific HRP injections in the posterior DMH and posterior hypothalamic nucleus show retrogradely labelled cells in the cerebellar nuclei. Therefore, direct cerebellar nuclei-posterior DMH and cerebellar nuclei-posterior hypothalamic connections are present. Nevertheless, the density of projections to these individual nuclei varied considerably between injections made into the posterior DMH and into the posterior hypothalamic nucleus. The density of projections was not the same, suggesting that the posterior DMH and posterior hypothalamic nucleus may modulate separate visceral functions.

Experimental studies have shown the involvement of the cerebellum in a variety of visceral responses (Sawyer et al. 1961; Achari et al. 1973). Miura & Reis (1969) and Chida et al. (1986) reported vasopressor and vasodepressor responses following stimulation of the medial cerebellar nucleus. Further, stimulation of the medial cerebellar nucleus produced cardiovascular and some behavioural responses (Chida et al. 1986; Oomura et al. 1989). It has been shown that medial cerebellar nucleus stimulation increases cerebral blood flow (Nakai et al. 1983). The consensus is that the medial cerebellar nucleus, via central pathways, initiates increases in cerebral blood (Ban, 1964;

Martner, 1975). This may be the result of direct cerebellar nucleus-hypothalamus connections as well as through the reticular nuclei. In contrast to the involvement of the medial cerebellar nucleus in cardiovascular responses, stimulation of the anterior interposed, lateral nucleus and posterior interposed nucleus of the cerebellum results in no such responses (Miura & Reis, 1970; Achari et al. 1973). Min et al. (1989) indicated that anterior interposed, lateral nucleus and posterior interposed nuclei projections to the hypothalamus may be excitatory in contrast to the inhibitory actions of the fastigiohypothalamic fibres. Haines et al. (1990) suggested the role of the interconnection between the anterior interposed, lateral nucleus and posterior interposed nuclei of the cerebellum and hypothalamus in mediating/regulating visceral activities, which are mutually coordinated with specific types of somatic responses.

Indirect multisynaptic cerebellovisceral interactions were thought to be primarily mediated through the reticular formation (Ban, 1964; Miura & Reis, 1969). Other brainstem relay nuclei mediating cerebellar input to the hypothalamus are reported as the dorsal motor nucleus of vagus, periaqueductal grey, the nucleus of the solitary tract, nucleus locus coeruleus and the raphe nucleus (Ban, 1964; Wallenberg, 1982). Dietrichs et al. (1985*b*) injected WGA-HRP complex into the hypothalamus and reported anterograde labelling in the lateral reticular nucleus. Furthermore, injection of WGA-HRP complex into the lateral reticular nucleus revealed the existence of labelled neurons in the lateral, dorsal, posterior and anterior hypothalamic areas and in the tuberomamillary, dorsomedial and periventricular nuclei of the hypothalamus. Chan-Palay (1977) and Somana & Walberg (1979) reported labelling in the dorsal motor nucleus and nuclei of solitary tract following injections into the cerebellar cortex and nuclei.

We also demonstrated that both the anterior and posterior DMH and the posterior hypothalamic nuclei have distinct connections with the lateral reticular nucleus and other reticular nuclei of the brainstem. Our experiments showed that both the posterior DMH and the posterior hypothalamic nucleus also received input from the solitary tract and vestibular nuclei, central grey, parabrachial nucleus and the trigeminal nucleus. These findings may suggest a possible route for indirect cerebellohypothalamic pathways.

Haines et al. (1984) postulated that cerebellar influences on visceral centres may be partially mediated through nucleohypothalamic and descending hypothalamic (solitary nucleus, dorsal motor nucleus and intermediolateral nuclei) circuits; fastigiohypothalamo-solitary and dentato-hypothalamo-dorsal motor of vagus are potential pathways. These observations suggest that visceral centres may be involved in feedback loops. Haines et al. (1984) suggested that the multisynaptic route via brainstem reticular nuclei may elicit slower and more generalised autonomic responses, whereas the direct route through cerebellar nucleohypothalamic circuit could give rise to more immediate and functionally more specific responses.

There is evidence of a general topographic pattern in the organisation of fibres extending between hypothalamus and cerebellum. The rostral and middle hypothalamic areas project to the medial cerebellar nucleus, whereas middle and more caudal areas project to the posterior interposed, anterior interposed and lateral cerebellar nuclei (Haines et al. 1984, 1990). Our results are in general agreement with the above findings.

We suggest that the anterior DMH may have a role in the indirect connections of the cerebellum via brainstem reticular nuclei, whereas the posterior DMH may have a role in both direct and indirect connections of the cerebellum. The cerebellum may contribute as a general modulator and coordinator of a wide variety of central nervous responses via direct or indirect projections to the DMH and posterior hypothalamic nucleus. The results of the present study provide clear evidence for the following. (1) There are distinct cerebellar nuclei-posterior DMH and cerebellar nuclei-posterior hypothalamic nucleus connections. The cerebellum can directly influence posterior DMH and posterior hypothalamus by way of this circuit, suggesting that the cerebellum plays an important role in the regulation of autonomic functions. (2) The brainstem reticular nuclei and other connections such as the solitary, trigeminal, vestibular and olivary nuclei may contribute in the indirect cerebellohypothalamic connections. (3) The DMH does not have uniform projections throughout its extent. The posterior DMH receives projections from the cerebellum, whereas the anterior DMH does not. (4) The densities of projections from the cerebellum to the posterior DMH and posterior hypothalamic nuclei are not the same. This suggests that the visceral functions modulated by these 2 hypothalamic nuclei cannot be the same.

The findings of this study support the hypothesis that the cerebellum may take part in modulating or influencing visceral responses via the above neural connections.

ACKNOWLEDGEMENTS

This work was supported by Marmara University Research Fund. We thank Yeşim Yimaz for her helpful comments on the paper.

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