THE PROJECTIONS OF THE CENTRAL NUCLEUS OF THE AMYGDALA TO THE DORSAL VAGAL COMPLEX A LIGHT MICROSCOPIC STUDY

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SUMMARY

This experiment was undertaken to determine and re examine the distribution of the descending projections from the central nucleus of the amygdala (CeA) to the dorsal vagal complex (DVC). This time it was accomplished by the anterograde and retrograde transport of Wheat Germ Agglutinin conjugated horseradish peroxidase(WGA-HRP), which was injected into the CeA using micropipettes. In no instances were injections of WGA-HRP in to the amygdala confined to the CeA. However it proved that from the effective injection uptake site only the CeA and bed nucleus of stria terminalis (BNST) projects from the higher brain centers to the dorsal medulla. The majority of efferent terminals from the CeA were observed in and around the dorsal motor nucleus of the vagus (DMV), into the medial sub nucleus of the nucleus of the tractus solitarius (NTSm) and commissural nucleus of the nucleus of the tractus solitarius (NTScom). In addition retrogradely labelled neurons were observed in the NTSm along its rostrocaudal extent.

INTRODUCTION

Johnston[1] described the CeA as phyllogenetically the oldest of the different nuclear groups of the amygdala. The CeA is further subdivided into four sub-divisions i.e. the medial, lateral, lateral capsular and ventral subdivisions[2, 3]. The CeA projects to and receive inputs from all the levels of the neuroaxis. The projections from the CeA terminate in a number of nuclei located in the thalamus, hypothalamus, midbrain, pons and medulla oblongata in a variety of species. Most of these sites are of significance in relation to the autonomic control of visceral function.

The DVC is a collective term, which includes the nucleus of the tractus solitarius (NTS), the DMV and the area postrema (AP). The NTS is the site for first synapse of the peripheral inputs from respiratory, alimentary and cardiovascular organs[4, 5,6,7,8,9]. Kalia and Sullivan[5] using cytoarchitectural criteria have divided the NTS of the rat into eight sub nuclei. The DMV is known to project to gastrointestinal, cardiovascular and pulmonary elements. The gastrointestinal tract receives parasympathic innervation from the DMV and sacral outflow[9, 10].

MATERIAL AND METHODS

A total of nine rats were used in this experiment. Micropipettes, with the tips with outer diameter of 25-35 micrometer (μm) were prepared. A 5% WGHRP solution was prepared by dissolving 1.0 mg WGAHRP conjugate in 20 microlitre (μl) of a 0.1% aqueous solution of neutral red.

The coordinates for WGA-HRP injection into the CeA was based upon

the atlas of Paxinos and Watson[11] and the results obtained from previous experiment[12].

Each experimental rat was anaesthetized and mounted in the Kopf stereotaxic frame. After exposing the skull, the periosteum was stripped from the surface of the parietal bone. A craniotomy was performed according to the coordinates for the CeA with the help of a dental drill.. Bone was removed until the dura mater was in view. The dura was incised using an iridectomy knife and the brain exposed. The micropipette, attached to a 1.0 µl Hamilton syringe containing 0.02 µl of the WGA-HRP, was lowered into the brain tissue to the point where CeA was located. The WGA-HRP solution was injected in two pulses of 0.01 µl at 5 minutes intervals and then micropipette was removed. Gel foam was placed over the burr hole and the incision was closed. After necessary antiseptic measures the animal was transferred to a clean cage for recovery.

Following surgery, after survival periods ranging from 72-96 hours, animals were perfused and fixed. The brains were removed and sectioned stereotaxically to produce three blocks consisting of forebrain, midbrain and the hindbrain. On the following day, frozen, transverse 40 µm thick serial sections were cut and processed for HRP histochemistry according to Mesulam[13] with slight modification.

Using a Zeiss microscopic, camera lucida drawings were made of the injection site in the amygdala from successful cases.

Sections of the medulla were examined with brightfield and darkfield illumination using a Zeiss microscope. The distribution of anterograde and retrograde labelling was mapped from representative sections. Using a Leitz photomicroscope, darkfield and brightfield photographs of representative sections of injection and projection sites were taken.

RESULTS

The CeA was encompassed within the injection site in 3 animals (DR 654, 655 and DR 740). In no case was it possible to restrict the injection exclusively to the CeA or even within the amygdaloid complex, although the centre of heaviest labelling involved the CeA (Fig. 1). In the other 6 cases, either the injection failed or the CeA was not involved in the injection site. In such cases, no transported label was observed in the DVC at any level. The brainstem labelling visible in case DR 740 was representative of the three successful cases. This case will be described in detail.

The injection site in DR 740 was centred on the CeA and involved all four subdivisions of the CeA[2,3] throughout the entire rostrocaudal extent of the CeA (Fig. 1, A to E). At the mid portion of the CeA, the tracer also involved the ventral portion of the caudate and putamen and globus pallidus (GP), the middle portion of the internal capsule, the dorsolateral portion of ventro postero lateral nucleus of the thalamus (VPL) and the reticular thalamic nucleus. Caudally the injection involved both the CeA and a portion of the internal capsule (Fig. 1, Sections B, C, D & E). Rostrally there was diffusion of tracer into the anterior amygdaloid area, the ventral portion of the substantia innominata (SI) and the region between the subcapsular stria medullaris thalami and the internal capsule (Fig. 1, Section A). Caudally this zone of diffusion spread to include portions of the lateral and basolateral amygdaloid nuclei, the dorsal part of the medial amygdaloid nucleus and some portion of the GP, caudate and putamen, reticular thalamic nucleus and VPL.

The criteria used to designate the effective injection uptake site and labelled structure were the same as explained by Awan & Rutherford[12] and Nauta et al[14]. Cross hatching indicates the region of effective uptake in Fig. 1, while the

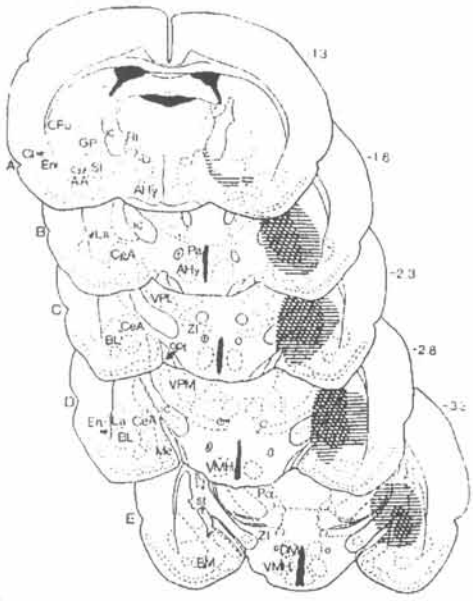

Fig. 1

Fig. 1 A series of camera lucida drawings of transverse sections through the forebrain showing the injection site in DR 740. Cross hatching indicates the area of effective uptake of WGA-HRP. The numbers given on right side correspond to the distance in mm from the bregma, as shown in the atlas of Paxinos and Watson[11].

ABBREVIATIONS

AA= Anterior amygdaloid area, AHy= Anterior hypothalamic nucleus, BL= Basolateral amygdaloid nucleus, BM= Basomedial amygdaloid nucleus, CeA= Central nucleus of the amygdala, Cl= Claustrum, CPu= Caudate & putamen, DM= Dorsomedial hypothalamic nucleus, En= Endopiriform nucleus, f= Fornix, GP= Globus pallidus, ic= Internal capsule, La= Lateral amygdaloid nucleus, Me= Medial amygdaloid nucleus, opt= Optic tract, Pa= Paraventricular nucleus of hypothalamus, Po= Posterior thalamic nuclear group, mt= Mamillothalamic tract, Rt= Reticular thalamic nucleus, SI= Substantia innominata, St= Stria terminalis, VMH= Ventromedial hypothalamic nucleus, VPL= Ventroposterior thalamic nucleus, lateral division, VPM= Ventroposterior thalamic nucleus, medial division, ZI= Zona incerta.

peripheral zone of the injection is indicated by parallel horizontal lines. The injection sites were similar in the other two successful cases except that in DR 655 the injection site did not involve the VPL and reticular thalamic nucleus. Although in all cases the injection sites extended beyond the boundaries of the CeA, data from the previous experiment[12] indicated that this is the only amygdaloid nucleus, which projects to the DVC.

WGA-HRP was both anterogradely and retrogradely transported to the DVC in DR 740. Retrogradely labelled neurons were located strictly ipsilateral to the injection site while anterograde transport was bilateral with an ipsilateral predominance up to the level of the obex. Rostral to the obex anterograde labelling was present ipsilaterally. The distribution of labelled structures is illustrated in Fig. 2 (camera lucida drawings), while Fig. 3 consists of a series of darkfield photomicrographs showing labelling at levels corresponding to the camera lucida drawings. For the sake of clarity, anterograde and retrograde labelling will be described separately.

ANTEROGRADE LABELLING

Anterograde labelling was observed in the DVC from a level 3.1 mm rostral to 1.3 mm caudal to the obex ipsilaterally, while contralaterally the amount of label was markedly light in sections caudal to the obex and totally absent rostral to the obex.

There was light anterograde label 1.0 mm caudal to the obex in the ventral portion of the NTScom. This label was heavier in the medial portion of the NTScom than in its lateral part, while the middle portion of the NTScom was free of label. Very light label was observed in the DMV and in the ventral subnucleus of the NTS. In the contralateral DVC, a similar distribution but less dense label was present.

At a level 0.6 mm caudal to the obex (at the caudal pole of the AP) anterograde

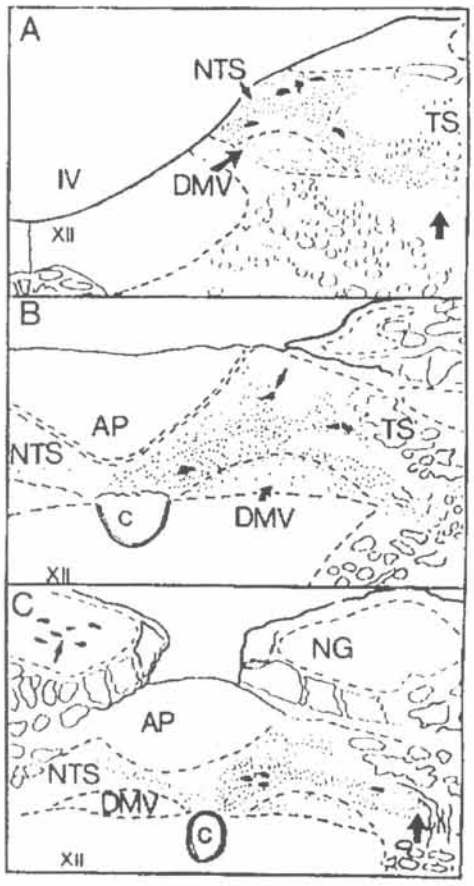

Fig. 2

Fig. 2 A series of camera lucida drawing of transverse sections through the medulla of DR 740. The distribution of anterograde labelling (stippling) and retrogradely labelled neurons is illustrated throughout the rostrocaudal extent of the DVC. Section A is rostral and C is caudal.

Anterograde labelling is indicated in the DMV by large and small curved arrows in sections A & B and in the ventral NTS by broad arrows in sections A & C. Small straight arrows indicate retrogradely labelled neurons in section B and anterograde labelling in the NTS in section A.

ABBREVIATIONS

AP= Area postrema, c= Central canal, CUm&l= Medial and lateral cuneate nucleus, DMV= Dorsal motor nucleus of vagus, IV= Fourth ventricle, FC= Fasciculus cuneatus, FG= Fasciculus gracilis, FLM= Medial longitudinal fasciculus, NG= Nucleus gracilis, NTS= Nucleus of tractus solitarius, NTST= Spinal trigeminal nucleus, NVm= Medial vestibular nucleus, NVs= Superior vestibular nucleus, TS= Solitary tract, TST= Spinal trigeminal tract, XII= Hypoglossal nucleus.

label was moderately dense and distributed in a pattern similar to that seen further caudally (Section C, Figs. 2 and 3). The dorsal portion of the NTScom and the lateral subdivision of the NTS contained very light label. Light amount of transported label was also present above the central canal and in the dorsal aspect of the DMV, while the DMV itself contained very light label (Section C in Figs. 2 and 3). In addition, the anterograde label was present in the ventral NTS (Section C, Figs. 2 and 3, broad arrows), in the ventrolateral subnucleus of the NTS and in the lateral medullary reticular formation (not illustrated).

At the level of the obex (Fig. 2 & 3 Section B) all the subnuclei[5] of the NTSm contained label except the central subnucleus and the subnucleus gelatinosus (the lateral part of the dorsomedial portion of the NTSm). The labelling was particularly heavy above the medial half of the DMV, while a few anterogradely labelled terminals were seen within the DMV (Section B, Figs. 2 and 3). Anterograde label extended into the dorsal reticular formation while passing through the ventrolateral nucleus of the lateral division of the NTS. On the contralateral side, the label was observed in similar regions but with markedly decreased intensity while with each successive rostral section, ipsilaterally, anterograde labelling increased in intensity.

In sections rostral to the obex (Section A, Figs. 2 and 3), the anterograde label was dense in the dorsomedial portion (the region which is adjacent to the floor of the IV ventricle) and the dorsal portion of the

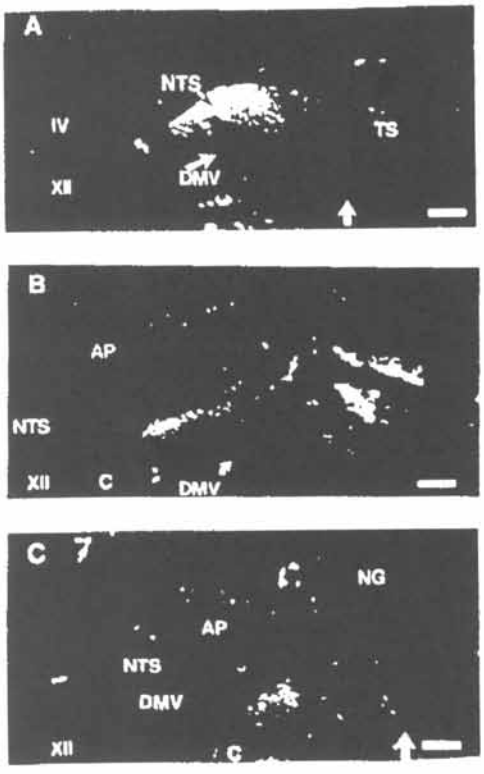

Fig. 3

Fig. 3 Anterograde and retrograde labelling in the DVC resulting from an injection of WGA-HRP into the CeA in case DR 740 is illustrated in this series of darkfield photomicrographs which corresponds to the levels illustrated in Fig. 2.

Bars= 200μm

All abbreviations as in Fig. 2.

NTSm, and lighter in the ventral subnucleus of the NTS (Section A, Figs. 2 and 3, broad arrow). The central, intermediate subnuclei and subnucleus gelatinosus were found to contain extremely light label or were entirely free of label.

Sparse anterograde labelling was observed throughout the length of the DMV. (Fig. 2, A to C). It was found that labelling was always heavier in the regions surrounding the DMV than in the nucleus it self.

RETROGRADE LABELLING

Retrogradely labelled neurons were observed in the NTS ipsilateral to injection site from the spinomedullary junction to 0.6 to 1.0 mm rostral to the obex. There were not more than three to five labelled neurons in any given section. Labelled cells appeared to be multipolar and were surrounded by anterogradely labelled terminals.

Caudal to the obex (Section C, Fig. 2 and 3), these retrogradely labelled neurons were present in the medial portion of the commissural nucleus and in the border zone between the DMV and rest of the NTS. In some sections, one or two retrogradely labelled cells were also observed in the ventral NTS just below the solitary tract (Fig. 2, Section C).

Rostral to the obex the retrogradely labelled neurons were mostly present in the medial subnucleus of the NTS, dorsal to the DMV, particularly in its dorsomedial portion (Section A, Figs. 2 and 3) and in the patch of anterograde label present between the central subnucleus of the NTS and the DMV. These neurons seemed to be scattered in the substance of the medial subnucleus and were observed up to 1.0-mm rostral to the obex in the NTS. In these rostral sections it was difficult to resolve whether these were retrogradely labelled neurons or neurons densely surrounded with the anterogradely transported label. No retrogradely labelled cells were found within the boundaries of the DMV at any level.

In addition to labelling in the DVC, a few retrogradely labelled neurons were found in the nucleus gracilis (NG) contralateral to the injection site (Section C, Figs. 2 and 3). A few lightly labelled neurons in the contralateral dorsal medullary reticular formation, just medial to the dorsal pole of the spinal trigeminal nucleus, were also observed. These neurons were most commonly noticed in sections caudal to obex.

A similar pattern of labelling in the DVC was displayed in the two other cases. In DR 655, no retrogradely labelled neurons were found in the NG.

In summary, injection of WGA-HRP into the CeA results in a distinct pattern of anterograde and retrograde labelling in the DVC: Bilateral, but predominantly ipsilateral, anterograde labelling located in the commissural, medial and ventral subnuclei of NTS caudally and most densely in the dorsomedial part of the NTSm and moderately in the DMV in sections rostral to the obex. The central, intermediate and gelatinosus subnuclei of the NTS contained either light label or no label at all.

A few retrogradely labelled neurons which were located principally in the medial part of the medial division of NTS, at the border zone between the DMV and NTS and, inconsistently, in the ventral NTS, ipsilateral to the injection site.

DISCUSSION

The purpose of this study was to reexamine the distribution of the descending projections from the CeA to the medulla.

In no instance were injections of WGA-HRP into the amygdala confined to the CeA. However, the results of the retrograde labelling studies[12] indicate that, in the areas contained within the injection sites, only the CeA and the BNST projects to the dorsal medulla. In cat, the BNST has been shown to project to the spinal trigeminal nucleus[15] as well as to the DVC. In the experiments reported here, no anterograde labelling was found in the spinal trigeminal nucleus following injection of WGA-HRP into the amygdala, suggesting that, if the rat and cat are similar in their projections, the BNST was not involved in the effective injection site. Moreover, in these cases in which the CeA was not involved in the injection, no projection to the DVC at any level was found. Thus, the anterograde labelling resulting from WGA-HRP injections into the amygdala is interpreted as representing the distribution of the projection from the CeA.

Projections from the CeA to the DVC were bilateral with an ipsilateral predominance in the caudal half of the medulla, while in the rostral medulla, the projections were found to be strictly ipsilateral. Caudally, these projections terminated in the commissural, medial and ventral subnuclei of the NTS bilaterally though with a strong ipsilateral predominance. Rostrally, ipsilateral to the injection site, these projections ended densely in the dorsomedial part of the medial subnucleus, moderately in the DMV and the rest of the medial subnucleus of the NTS, and extended ventrally through the ventral nucleus into the lateral medullary reticular formation. The central, intermediate and gelatinous subnuclei of the NTS were free of any terminal labelling in the rat.

Retrogradely labelled neurons were present ipsilaterally in the NTS at a level from the spinomedullary junction to 1.0 mm rostral to the obex. These neurons were multipolar, but smaller in size than the DMV neurons and were located in the medial subnucleus of the NTS.

In an earlier study, van der Kooy et al[16] also injected WGA-HRP in the CeA in rat. Their injection sites were comparable to those reported here. The projections to the DVC which they described are in agreement with the finding presented here except that in the present study dense anterograde labelling was observed in the medial portion of the medial subnucleus of the NTS in sections caudal to the obex (compare Fig. 12 from van der Kooy et al[16], with Figs. 2 & 3, Sections B and C, present study). The anterograde labelling in the caudal NTS on the contralateral side was also mentioned by van der Kooy et al[16]. This labelling may possibly be the result of collateralization of the axons of CeA neurons at this level,

because some anterograde label was found distributed above the central canal in the NTS (COM). It should be pointed out that injection of WGA-HRP into the DVC produced only ipsilateral retrograde labelling (A&R) in the amygdala, suggesting the need for further studies using a variety of different tracers.

Autoradiographic studies done in the cat[17], rabbit[18] and monkey[19] yielded similar results to those reported here. However in rabbit, the study by Schwaber et al[18] showed a denser labelling in the DMV and the ventral NTS than is reported here, which could be the result of different techniques and/or due to difference in species. Observations made by Price and Amaral[19] in monkey, that DMV was surrounded by anterograde terminals and labelling in the DMV was light is in agreement with this study and other anterograde experiments. HRP studies in cat and monkey[20] yielded results, which are similar to my observations.

Phaseolus vulgaris leucoagglutinin (PHA-L) was used by Danielsen et al[21] to examine the subcortical projection field of the CeA in the rat. The description by Danielsen regarding the CeA projections to the DVC is generally in accord with present observations, except that PHA-L produced dense axonal labelling in the DMV in rostral regions of the DVC, while intermediate subnucleus of the NTS was also found to contain label. In this study the amount of label observed in the DMV was moderate and light respectively rostral and caudal to the obex, while very light label was observed in the intermediate subnucleus of the NTS. Danielsen also observed very light contralateral labelling in the NTS/DMV at all levels, while in my study contralateral label was present only up to the level of obex. However, due to the restricted injection sites which PHA-L injection produce, the Danielsen study was able to clearly show that it is the medial part of CeA which predominantly projects to DVC. The discrepancy regarding the ratio of labelling observed by Danielsen et al[21] can be attributed to the sensitive nature of the PHA-L in comparison to WGA-HRP.

The distribution of retrogradely labelled neurons which was observed in the NTSm in this experiment had also been reported in retrograde studies by Ricardo and Koh[22], van der Kooy et al[16] and Riche et al[23] in rat and by Volz et al[24] in cat. In 1981, Hopkins[20] also acknowledged the presence of retrogradely labelled neurons in the DVC, when HRP was injected into the amygdala of the cat. The HRP injections in the CeA of rat by Ricardo and Koh[22] and in cat by Volz et al[24] were more circumscribed than those reported here or in van der Kooy et al[16] work. In present study, labelled neurons were observed up to a rostral level of the NTSm, where the DVC is juxtaposed to the floor of the IV ventricle. The only difference in the present observations and those reported elsewhere is that, the three other studies mention the presence of labelled neurons in the NTS only up to the level of the AP. However, Riche et al[23] injected a protein gold complex in the CeA and reported the presence of retrogradely labelled neurons in the NTS throughout its rostrocaudal extent ipsilaterally. The difference in the distribution of retrogradely labelled neurons may be related to the superior sensitivity of the WGA-HRP used in this study. In the studies mentioned above mostly HRP was used as the tracer. In all these studies, including this one, no retrogradely labelled neuron was found in the DMV.

No afferent labelling was observed in the NG at any level. This observation indicates that the CeA does not project to this nucleus. However the infusion of WGA-HRP in the thalamic nucleus resulted in retrograde labelling of neurons in the contralateral NG in DR 740 and DR 654.

These experimental results were helpful in guiding the EM experiments to be considered next. These findings indicated that the best places to look for terminals of CeA neurons at the EM level would be in and around the DMV, into the NTSm and NTScom, because these are the regions where the majority of efferent projections from the CeA were observed in this light experiment.

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