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Research article

Amygdalotrigeminal projection in the rat: An anterograde tracing study

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SUMMARY

Previous neurophysiological studies have demonstrated that the amygdala has a direct influence upon trigeminal motoneuron activity. The existence of a direct amygdalotrigeminal pathway in rats was proved by anterograde tracing with the neuroanatomical tracer, biotinylated dextran amine (BDA). After ipsilateral BDA application to the central nucleus of the amygdala (AmCe), widespread ipsilateral projections emerging from its medial subnucleus were traced to the trigeminal brainstem nuclear complex, including the principal sensory (Pr5) and mesencephalic trigeminal nucleus (Me5), and their premotoneurons and interneurons, located in the supratrigeminal, intertrigeminal and peritrigeminal nuclei. Sparse BDA-labeled axons and their terminals were also distributed in the contralateral Pr5, interpolar and caudal subnuclei of the spinal trigeminal nucleus. The central lateral amygdaloid nucleus gives rise to a light ipsilateral projection to the pontine part of the Me5. The present data indicate that AmCe sends massive efferents to the trigeminal nuclei in the brainstem, wherein its medial subnucleus sends the major input to them. The medial amygdaloid nucleus sparsely innervates Me5 neurons, specifically those located in its mesencephalic portion, while basomedial and basolateral efferents do not target the trigeminal nuclear complex. These results suggest that the amygdaloid input may modulate the activity of trigeminal sensory and motor neurons and, thus, the amygdala is possibly involved in the control of masticatory behavior.

1. Introduction

The human amygdala (Am) is an important brain structure, located deep within the ventromedial temporal lobe, ventral to the caudolateral striatum and the pallidum. The amygdaloid nuclear complex consists of several structurally and functionally distinct nuclei, generally divided on the basis of cytoarchitectonic, hodological, histochemical, and immunohistochemical studies, into a corticomedial complex and a basolateral complex. The latter is evolutionarily newer and comprises the lateral, basolateral and basomedial amygdaloid nuclei, while the former is phylogenetically older and encompasses the centromedial and cortical nuclei (reviewed in Amaral et al., 1992; McDonald, 1992; De Olmos et al., 2004). The Am is involved in the modulation of neuroendocrine functions, visceral efferent motor mechanisms, and in a vast range of normal behavioral functions and psychiatric con-

Abbreviations: ACo, anterior cortical amygdaloid nucleus; Am, amygdala; AmBl, basolateral nucleus of the amygdala; AmBm, basomedial nucleus of the amygdala; AmCe, central nucleus of the amygdala; AmLa, lateral nucleus of the amygdala; AmMe, medial nucleus of the amygdala; Astr, amygdalostriatal transition area; BDA, biotinylated dextran amine; CeC, central amygdaloid nucleus, capsular part; CeL, central amygdaloid nucleus, lateral division; CNS, central nervous system; DEn, dorsal endopiriform nucleus; I, intercalated nuclei of the amygdala; I5, intertrigeminal nucleus; LC, locus coeruleus; LPB, lateral parabrachial nucleus; LV, lateral ventricle; MGP, medial globus pallidus; Me5, mesencephalic trigeminal nucleus; MePV, medial amygdaloid nucleus anteroventral part; MPB, medial parabrachial nucleus; Mo5, motor trigeminal nucleus; opt, optic tract; PAG, periaqueductal gray; Pa5, paratrigeminal nucleus; Pir, piriform cortex; PLCo, posterolateral cortical amygdaloid nucleus; P5, peritrigeminal nucleus; Pr5, principal sensory trigeminal nucleus; Pr5DM, principal sensory trigeminal nucleus, dorsomedial part; scp, superior cerebellar peduncle; Sp5, spinal trigeminal nucleus; Sp5C, spinal trigeminal nucleus, caudal part; Sp5I, spinal trigeminal nucleus, interpolar part; Sp5O, spinal trigeminal nucleus, oral part; st, stria terminalis; Su5, supratrigeminal nucleus; 4V, 4th ventricle.

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[1] Dr. Kamen Usunoff passed away unexpectedly on February 28, 2009 while on a study stay in Rostock, Germany during the preparation of the manuscript. He contributed equally to this paper and should be considered a first co-author.

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ditions (for comprehensive reviews see Ben-Ari, 1981; Aggleton, 1992; Aggleton and Saunders, 2000), and has a wide variety of afferent and efferent connections throughout the central nervous system (CNS; Pitkänen, 2000). In particular, neuroanatomical studies in rats have demonstrated that Am sends efferents to a variety of brainstem regions including substantia nigra/ventral tegmental region, central gray, parabrachial nuclei, dorsal vagal complex and ventrolateral medulla (Hopkins, 1975; Price et al., 1987; Danielsen et al., 1989; Wallace et al., 1992; Tsumori et al., 2010).

The trigeminal brainstem nuclear complex in the rat, which receives somatosensory input from the orofacial region of the head, is located throughout the whole dorsolateral length of the brainstem, extending from the midbrain to the upper cervical spinal cord. The structure and connections of this complex in humans has been the subject of comprehensive reviews by Usunoff et al. (1997), and Waite and Ashwell (2004). It is an important sensorimotor center that is comprised sensory and motor nuclei. From rostrally to caudally, the trigeminal sensory nuclei include the mesencephalic trigeminal nucleus (Me5), the main or principal sensory nucleus (Pr5), and the spinal trigeminal nucleus (Sp5), which is further subdivided into three subnuclei, the oralis (Sp5O), the interpolaris (Sp5I) and the caudalis (Sp5C) (Olszewski, 1950; Olszewski and Baxter, 1954; Darian-Smith, 1973; Waite, 2004). The distinct trigeminal motor nucleus (Mo5) is located medially to the main sensory nucleus in the pontine tegmentum. Brainstem trigeminal nuclei also include the supratrigeminal nucleus (Su5) on the dorsomedial pole of Pr5, the intertrigeminal nucleus (I5), which lies between Pr5 and Mo5 (Waite, 2004), and the peritrigeminal nucleus (P5), located lateral and ventral to the trigeminal tract at the level of the Sp5I (Paxinos and Watson, 1998). The paratrigeminal nucleus (Pa5) has traditionally been thought of as a separate sensory component (Chan-Palay, 1978) but, at least in humans, it has been considered as another subdivision of the spinal nuclei (see Usunoff et al., 1997). Motor and premotor neurons, located in the Mo5, Su5 and I5, respectively, are associated with masticatory reflexes and the control of jaw movements (see Travers, 2004 for a recent review).

Previous physiological investigations have shown that Am has a direct influence upon trigeminal motoneuron activity because electrical stimulation of the central amygdaloid nucleus can produce rhythmic jaw movements in rats (Sasamoto and Ohta, 1982; Ohta, 1984). Besides, it has been demonstrated that long-lasting stimulation of the basal amygdaloid nucleus causes a delayed inhibition of the masseter reflex (Bobo and Bonvallet, 1975), mediated through the pars oralis of the Sp5 (Bonvallet and Bobo, 1975). This raises the intriguing possibility of a direct amygdalotrigeminal connection. However, none of these studies reveals the existence of monosynaptic projections from Am to trigeminal motoneurons. To date, evidence has only been provided for an amygdalofugal projection to the mesencephalic trigeminal nucleus in the rat (Krettek and Price, 1978; Post and Mai, 1980; Price and Amaral, 1981). In addition, an indirect descending pathway from the central amygdaloid nucleus via the pontine reticular formation to the contralateral Mo5 and ipsilateral supratrigeminal region has been traced in rats (Takeuchi et al., 1988a,b). Recently, a bilateral disynaptic pathway from the rat central amygdaloid nucleus to the Mo5 via the parvicellular reticular formation of the medulla oblongata, where many trigeminal premotor neurons are located, has been reported as well (Yasui et al., 2004; Tsumori et al., 2010). Nevertheless, the existence and organization of a direct amygdalotrigeminal pathway remains to be determined and, therefore, we decided to re-examine its real presence.

In the present study, we report efferent projections to the trigeminal nuclei in the brainstem from the amygdaloid nuclear complex, a key structure of the limbic system, studied by anterograde axonal tracing with the anterograde neuroanatomical tracer biotinylated dextran amine (BDA).

2. Materials and methods

The experiments in this study were carried out on 21 adult Wistar rats of both sexes, weighing 220–260 g. The surgical procedures involving animals and their care were conducted in conformity with the standards for animal experiments according to a protocol (Nr. 1414/2007) approved by the Animal Care and Use Committees at our universities and were consonant with the guidelines established by the NIH.

The animals were anesthetized with Thiopental (50 mg/kg, i.p.) and then placed on a stereotaxic apparatus (David Kopf, Tujunga, CA) in the flat skull position. Stereotaxic coordinates of the amygdaloid nuclei and the list of abbreviations used in the text and figures were obtained from the atlas of Paxinos and Watson (1998). Under aseptic conditions small craniotomies were performed. In four Am nuclei (central nucleus, AmCe – 10 rats; medial nucleus, AmMe – 6 rats; basomedial nucleus, AmBm – 3 rats; and basolateral nucleus, AmBl – 2 rats) 0.25–0.5 μl 10% BDA (10,000 MW; Molecular Probes Europe BV, Leiden, The Netherlands) dissolved in 0.1 M phosphate buffer (PB; pH 7.4) was injected using a dorsal approach. Multiple unilateral injections were made by pressure over a period of 30–60 min using a pico-spritzer through a glass micropipette (20–50 μm tip diameter) which was attached to a Hamilton microsyringe (Hamilton Co., Reno, NV). At the end of the injection, the pipette was held in place for 15 min to insure that the injected tracer had been absorbed into the tissue and to reduce the possibility of its spread. The site of microinjection was verified on coronal sections. After survival time of 7–14 days, the rats were deeply anesthetized and perfused transcardially with phosphate buffered saline (PBS), followed by 500 ml of 4% paraformaldehyde in PB. The brains were removed and postfixed overnight in the same fixative, sliced in the coronal plane and immersed in 0.5% paraformaldehyde in PBS containing 20% sucrose at 4 °C. Serial sections were cut at a thickness of 40 μm on a Reichert Jung freezing microtome, collected in a free-floating state in PBS and then processed for tracer histochemistry. A commercial avidin–biotin–HRP complex (ABC) kit was used to visualize BDA (Vectastain ABC Kit, Vector Laboratories Inc., Burlingame, CA). Briefly, the sections were preincubated in PBS containing 0.1% bovine albumin (fraction V; Sigma Chemical Co., St. Louis, MO) for 20 min, and rinsed in PBS for 30 min. Subsequently they were incubated in the avidin-coupled biotinylated HRP solution for 45–60 min, and rinsed again in PBS for 30 min. The reaction product was developed with 0.06% 3,3^{I}-diaminobenzidine (Sigma, St. Louis, MO) and 0.02% H_2O_2 in Tris buffer (0.05 M, pH 7.6) for 10–15 min in the dark. The sections were afterward rinsed in distilled water, mounted onto chrome alum-gelatin-coated slides and air dried overnight. Finally, the sections were counterstained with 0.025% cresyl violet, dehydrated, and coverslipped with Entellan (Merck, Darmstadt, Germany). The slides were then examined in a Zeiss Axioplan 2 research microscope and selected areas were photographed with an AxioCam MRc digital camera. Adobe Photoshop CS3 (Adobe Systems Inc., San Jose, CA) was used to adjust contrast and brightness of all photomicrographs.

3. Results

3.1. AmCe injection site (10 rats)

In 10 cases studied the injection site involved the central nucleus of the amygdala (AmCe). In five animals in which a larger quantity of BDA was delivered (0.5 μl), three of the cases had injections in the AmCe (Fig. 1A and C) without spread of the tracer into surround-