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DISTRIBUTION OF HYPOTHALAMIC, HIPPOCAMPAL AND OTHER LIMBIC PEPTIDERGIC NEURONAL CELL BODIES GIVING RISE TO RETINOPETAL FIBERS: ANTEROGRADE AND RETROGRADE TRACING AND NEUROPEPTIDE IMMUNOHISTOCHEMICAL STUDIES

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Abstract—In our present work utilizing the retrograde or anterograde transport of tracers (biotinylated dextran amine and Fluorogold, respectively) we have provided direct evidence for the cells of origin of the limboretinal pathway in rats and their termination in the retina using light microscopic approach. Administration of biotinylated dextran amine into the vitreous body resulted in nerve cell body labeling in several structures: the supraoptic and paraventricular nuclei, the hippocampus (CA1, CA3), the dentate gyrus, the indusium griseum, the olfactory tubercle, and the medial habenula, all of them belong to the limbic system. We estimated that the total number of retrogradely labeled cells is 1495±516. We have seen fiber labeling in the retinorecipient suprachiasmatic nucleus and in the primary visual center, the lateral geniculate body, but labeled nerve cell bodies in these structures were never seen. Iontophoretic application of Fluorogold into the hippocampal formation, where the major part of the biotinylated dextran amine-labeled cell bodies was observed, resulted in labeled fibers in the optic nerve and in the retina indicating that the retrogradely labeled cells in the hippocampus and the dentate gyrus among others are the cells of origin of the centrifugal visual fibers. Sections showing biotinylated dextran amine labeling were stained for vasoactive intestinal polypeptide, pituitary adenylate cyclase activating polypeptide or luteinizing hormone-releasing hormone immunoreactivity using immunohistochemistry. Some biotinylated dextran amine-labeled cells also showed vasoactive intestinal polypeptide, pituitary adenylate cyclase activating polypeptide or luteinizing hormone-releasing hormone immunoreactivity. We conclude that the limboretinal pathway exists and that the cells of origin are partially vasoactive intestinal polypeptide, pituitary adenylate cyclase activating polypeptide or luteinizing hormone-releasing hormone immunoreactive. © 2006 Published by Elsevier Ltd on behalf of IBRO.

Key words: limboretinal pathway, tracing, VIP, PACAP, LHRH.

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Abbreviations: BDA, biotinylated dextran amine, 10,000 MW; CA1, CA1 region of the hippocampus; CA3, CA3 region of the hippocampus; DAB, diaminobenzidine tetrahydrochloride; DAPI, 4,6-diamidine-2-phenylindole dihydrochloride; DG, dentate gyrus; FB, Fast Blue; FG, Fluorogold; H, habenular complex; HRP, horseradish peroxidase; IG, indusium griseum; INL, inner granular layer; IPL, inner plexiform layer; KPBS, potassium phosphate-buffered saline; LGB, lateral geniculate body; LHRH, luteinizing hormone-releasing hormone; PACAP, pituitary adenylate cyclase activating polypeptide; PFA, paraformaldehyde; PV, paraventricular nucleus; SCH, suprachiasmatic nucleus; SO, supraoptic nucleus; Tu, olfactory tubercle; VIP, vasoactive intestinal polypeptide.

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It is well established that the retinal ganglion cells, other than the primary visual center, send their axons or axon collaterals to the hypothalamic nuclei. These fibers form the retinohypothalamic pathway (Hendrickson et al., 1972; Moore and Lenn, 1972). The major neurotransmitter of this pathway is glutamate, although other transmitters and neuropeptides have also been demonstrated including pituitary adenylate cyclase activating polypeptide (PACAP) (Köves et al., 1996; Hannibal et al., 1997). The major part of these fibers terminates in the suprachiasmatic nucleus (SCH); however, other retinorecipient areas in the hypothalamus have been demonstrated as well (Mai and Junger, 1977; Mai, 1979; Silver and Brand, 1979; Kita and Oomura, 1982; Levine et al., 1994).

Since the description of the olivocochlear bundle by Rasmussen (1946), both anatomical and physiological evidence appears to demonstrate the existence of centrifugal fibers within most of the major sensory pathways. Previous to the use of tracing techniques the presence of these fibers in the avian retina and optic nerve was reported by Ramon y Cajal (1893) and Dogiel (1895). Later, retinopetal projections were described in all vertebrates, including human (Wolter and Knoblich, 1965; Brooke et al., 1965; Wolter, 1978; Repérant et al., 1989; Uchiyama, 1989; Ward et al., 1991). Several structures were proposed to be the origin of these fibers: the reticular formation of the brain stem (Granit, 1955), the superior colliculus (Polyak, 1957) and the lateral geniculate nucleus (Wolter and Lund, 1968).

In lower vertebrates many data have been accumulated about the existence of the centrifugal visual fibers during the last century. The major source of these fibers in birds is the isthmo-optic nucleus (Cowan and Powell, 1962). In snakes the nucleus of the ventral supraoptic decussation is known to send centrifugal visual fibers to the retina, a higher number to the contralateral than to the

ipsilateral eye (Halpern et al., 1976). In chameleon, Hassni et al. (1997) described retinopetal nuclei in the ventromedial tegmental region of the mesencephalon and the ventrolateral thalamus of the diencephalon. In their experiments, rhodamine and horseradish peroxidase (HRP) were used as retrograde tracers. In turtles, after intraocular injection of wheat-germ agglutinin (WGA) or HRP Schnyder and Kunzle (1983) described retrogradely labeled neurons in the mesencephalic reticular area lying between the trochlear and isthmic nuclei. Their number was small and they were predominantly found contralateral to the injected side.

In mammals centrifugal visual fibers have also been observed. With the use of large molecule retrograde tracers such as HRP injected into the vitreous body of rats, Itaya (1980) and Itaya and Itaya (1985) demonstrated neurons in the pretectal area and in the periaqueductal gray matter which send their axons to the retina, although they did not mention labeled cells in the hypothalamus or in other extrahypothalamic structures. Other researchers have found retrogradely labeled cells in the caudal mesencephalon, in the medial pretectal area and in the oculomotor nucleus of the same species (Hoogland et al., 1985; Villar et al., 1987; Labandeira-Garcia, 1988). In another experiment after an injection of HRP into the vitreous body of dogs, retrogradely labeled cells were observed in the ventral hypothalamus (Terubayashi et al., 1983), while in monkey a fluorescent tracer, Fast Blue (FB), was transported from the vitreous body to the SCH and the arcuate nuclei (ARC) (Bons and Petter, 1986).

The termination of centrifugal visual fibers in lower vertebrates has been exhaustively studied by researchers (Dowling and Cowan, 1966; Repérant et al., 1989; Uchiyama, 1989; Vesselkin et al., 1989; Miceli et al., 1999). On the bases of their results it is clear that the number of centrifugal visual fibers is limited; however, in the retina they show a rich arborization. With the use of electron microscope Dowling and Cowan (1966) described the termination of centrifugal visual fibers of pigeon. The endings are most often found along the outer margin of the inner plexiform layer (IPL) and between the lowermost cells of the inner nuclear layer. The majority of the terminals have contact with the basal part of the amacrine cells. Their observation is very similar to that of Ramon y Cajal (1911) using silver impregnation. It was also demonstrated that the major part of the centrifugal fibers is glutamatergic, GABA-ergic (Rio et al., 1993, 1996, 2003) or serotoninergic (Villar et al., 1987), although neuropeptides were also observed in these fibers. Luteinizing hormone-releasing hormone (LHRH) fibers were seen in fish (Münz et al., 1982; Stell et al., 1984; Grens et al., 2005), frogs (Wirsig-Wiechmann and Basinger, 1988), crocodile (Medina et al., 2005) and in rats (Santacana et al., 1996). In fish the LHRH fibers belong to the olfactoretinal nerve whose mammalian counterpart is the terminal nerve. In rats the origin of these fibers has not been demonstrated.

In our previous study (Fógel et al., 1997) we described bilateral fiber bundle immunoreactive to vasoactive intestinal polypeptide (VIP) in rat projecting into the optic chiasm and the optic nerves from the hypothalamus. We supposed that these fibers may originate from the forebrain, partially from the supraoptic (SO) and the paraventricular (PV) nuclei. This was based on the observation that after removal of the eyes (enucleation), a few VIP immunoreactive cell bodies appeared in these nuclei, although in intact rats VIP cells were never seen in these locations. The accumulation of VIP immunoreactive material in these cell bodies might be caused by the interruption of their axons by the enucleation. Five to six months after enucleation, the fibers originating from the retina are completely degenerated. In these animals *Phaseolus vulgaris* leucoagglutinin (PhA-L) was administered iontophoretically into the SO or the PV region and transported into the optic nerve by the surviving fibers; however, the number of the labeled fibers was very limited. This observation suggested that some of the cells sending centrifugal visual fibers to the eye may reside in these regions, but the majority have to be looked for in other parts of the CNS.

The main aim of the present work is to provide direct evidence for the localization in the forebrain of the cell bodies that give rise to the centrifugal visual fibers. To do this we utilized the retrograde transport of a tracer injected in the vitreous body and the anterograde transport of an iontophoretically applied tracer to the locations where the majority of these cells were expected.

The presence of LHRH, VIP and PACAP immunoreactive fibers in the optic chiasm and optic nerves is well established. There is also evidence that PACAP immunoreactive fibers may belong to the retinal ganglion cells (Köves et al., 1996; Hannibal et al., 1997), therefore they are retinofugal, although some fibers may also be retinopetal. In paddy-birds, LHRH immunoreactivity was also observed in the ganglion cells (Fukuda et al., 1982); however, LHRH immunoreactivity was also found in retinopetal fibers of various species as described above. VIP immunoreactive fibers in the optic nerve seem to be retinopetal because the retinal ganglion cells are not VIP immunoreactive (Casini and Brecha, 1991).

On the basis of the abovementioned results we propose that a part of the PACAP, VIP and LHRH immunoreactive fibers belongs to cells that give rise to retinopetal (centrifugal visual) fibers and that these cells reside in the forebrain. To demonstrate this, forebrain sections containing BDA labeled cell bodies were also stained for PACAP, VIP or LHRH immunoreactivity showing the chemical character of the cells sending the centrifugal visual fibers to the eyes.

EXPERIMENTAL PROCEDURES

Experimental animals

Adult Sprague–Dawley male rats (2–4 months old) were used in our experiments. The animals were kept in a light- and temperature-controlled vivarium (lights on at 06:00 h and off at 18:00 h, temperature 22 °C±2). They were provided with standard laboratory chow and water *ad libitum*. All interventions, including the injection of the tracer into the vitreous body of the eye, the iontophoretic administration of the tracer into the forebrain regions and the final perfusion, were carried out under general anesthesia using ketamine-hydrochloride (75 mg/100 g bw) (Sigma, St. Louis,