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The cocaine- and amphetamine-regulated transcript, calbindin, calretinin and parvalbumin immunoreactivity in the medial geniculate body of the guinea pig

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ABSTRACT

The purpose of this study was to describe the distribution and colocalization of cocaine- and amphetamine-regulated transcript (CART) and three calcium-binding proteins (calbindin, calretinin and parvalbumin) in each main division of the medial geniculate body (MGB) in the guinea pig. From low to moderate CART immunoreactivity was observed in all divisions of the MGB, although in most of its length only fibers and neuropil were labeled. A small number of CART immunoreactive somata were observed in the caudal segment of the MGB. The central parts of all divisions contained a distinctly smaller number of CART immunoreactive fibers relative to their outer borders, where CART fibers formed patchy clusters. As a whole, the intense CART immunoreactive borders formed a shell around the weakly CART labeled core. Double-labeling immunofluorescence showed that CART did not colocalize with either calbindin, calretinin or parvalbumin, whose immunoreactivity was predominantly restricted to perikarya. The distribution pattern of calretinin was more similar to that of calbindin than to that of parvalbumin. Calretinin and calbindin exhibited higher immunoreactivity in the medial and dorsal divisions of the MGB, where parvalbumin staining was low. In general, although parvalbumin exhibited the weakest immunoreactivity of all studied Ca^{2+} binding proteins, it was most highly expressed in the ventral division of the MGB. Our results indicate that CART could be involved in hearing, although its immunoreactivity in the medial geniculate complex was not as intense as in other sensory brain regions. In the guinea pig the heterogeneous and complementary pattern of calbindin, calretinin and parvalbumin is evident, however, the overlap in staining appears to be more extensive than that seen in other rodents.

Introduction

The medial geniculate body (MGB) of mammals is a part of the thalamus, which serves as one of the sensory processing centers. The MGB is the main relay structure of the primary auditory pathway that ascends from the inferior colliculus and terminates in the primary auditory cortex. Anatomical and physiological studies have shown that the MGB consists of three primary divisions: the dorsal nucleus (MGd), the ventral nucleus (MGv) and the medial nucleus (MGm) (Winer, 1984; Winer et al., 1988; Winer and Wenstrup, 1994; Najdzion et al., 2011). These three nuclei display a diverse array of reciprocal corticothalamic connections, mostly among auditory brain regions, however, the MGd and MGm also receive nonauditory afferents (Zimny et al., 1981; Middlebrooks and Zook, 1983; Henkel, 1983; Winer and Morest, 1983; Oliver, 1984; Niimi et al., 1984; LeDoux et al., 1985; Rouiller et al., 1985; Rouiller and De Ribaupierre, 1985; LeDoux et al., 1987; Clerici and Coleman, 1990; Mello et al., 1992). The MGB utilizes a wide variety of neurotransmitters, such as GABA, acetylcholine, somatostatin (the latter can also act as a neuromodulator) and stains positive for different calcium binding proteins (CaBPs) (Winer and Larue, 1988; Winer et al., 1992; Münkle et al., 2000; Cruikshank et al., 2001; Pego-Reigosa et al., 2001; Olucha-Bordonau et al., 2004; Lu et al., 2009).

Cocaine- and amphetamine-regulated transcript (CART) is a relatively novel putative neurotransmitter widely distributed throughout the central and peripheral nervous systems, as well as in endocrine cells in the pituitary and adrenal glands (Koylu et al., 1997, 1998). In the brain the distribution of CART seems to be wide, but selective. CART is highly expressed in regions of the brain associated with the regulation of food intake, such as the paraventricular nucleus of the hypothalamus, ventromedial

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nucleus, dorsomedial nucleus, lateral hypothalamus, arcuate nucleus and the lateral septum (Koylu et al., 1997, 1998; Janzsó et al., 2010). It is also found in brain regions associated with reward, reinforcement and drug abuse, including the ventral tegmental area and the nucleus accumbens (Jaworski and Jones, 2006). Furthermore, CART peptides were identified in the preoptic area, which is a hypothalamic region strictly connected with reproductive and maternal behaviour (Bogus-Nowakowska et al., 2011, 2012). Finally, CART mRNA is highly expressed in limbic and sensory related brain regions, namely the cortex, the amygdala (central, cortical and medial nuclei), the hippocampus (uncal and dentate gyrus) and in many thalamic nuclei (Hurd and Fagergren, 2000). CART mRNA is present in mitral and tufted cells of the olfactory bulb, and in retinal ganglion cells (Couceyro et al., 1997). Koylu et al. (1998) showed that the presence of CART peptides in the retina and olfactory areas was consistent with the in situ hybridization findings. The inner plexiform layer of the olfactory bulb exhibits a very low density of stained fibers, while the outer plexiform layer contains numerous immunoreactive cells. The mitral cells of the main olfactory bulb also consistently stain positive for CART, and the accessory olfactory bulb shows a high level of CART immunoreactivity. Additionally, CART is found throughout primary olfactory cortical areas, including anterior olfactory nucleus, taenia tecta, olfactory tubercle, piriform cortex and indusium griseum. In the retina Koylu et al. (1998) found there are stained cell bodies in the ganglion cell layer. Dense CART immunoreactivity is also found in the inner plexiform layer. These results show that CART is abundant in regions of the brain involved in olfaction and vision, suggesting CART involvement in sensory information processing (Couceyro et al., 1997).

Although there are few reports on CART immunoreactivity in geniculate bodies (Koylu et al., 1998; Hunter et al., 2005), they are very general and do not contain data on the guinea pig. The purpose of the present study was to describe the distribution of CART in each main division of the MGB in this species, and specifically to determine if this putative neurotransmitter is present in the main relay structure of the auditory pathway as abundantly as in the brain regions associated with olfaction and vision. We also aimed to determine whether or not CART colocalizes with calbindin, calretinin and parvalbumin, which are calcium binding proteins (CaBPs) that serve as useful markers of different neuronal populations in the brain and which have important roles in maintaining intracellular calcium homeostasis (Baimbridge et al., 1992). Comprehensive information on CaBPs immunoreactivity in the medial geniculate body of the guinea pig also has not been reported. Since the guinea pig has become a popular model organism for hearing research, we believe our new data will help in elucidating the complex structure and function of the auditory system. Furthermore, it will also provide a better understanding of the parallels and differences among species from different orders.

Material and methods

Animals and tissue processing

The study was performed on 6 sexually mature guinea pigs (strain: Dunkin-Hartley, obtained from the Research Institute of the Polish Mothers' Health Centre in Lodz, Poland). All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. Surgical procedures followed the guidelines established by the Animal Care and Use Ethical Committee of the University of Warmia and Mazury. All procedures were carried out in accordance with EU Directive 2010/63/EU for animal experiments. All animals were anaesthitized with lethal dose of sodium pentobarbital (Morbital, Biowet, Poland; 2 ml/kg b.w.) and perfused intracardially with 4% paraformaldehyde. Next, the brains were removed from the skulls and postfixed for 30 min in the same fixative. Brains were washed twice in 0.1 M phosphate buffer and then cryoprotected in sucrose. Frozen brains were cut into 10 μm coronal plane sections on a cryostat.

Immunohistochemistry

Tissue sections were processed for routine single- and double-labeling immunofluorescence using mouse monoclonal antibodies against CART (1:6000; code MAB 163, R&D Systems, USA) or rabbit polyclonal antibodies against CART (1:8000; code H-003-61, Phoenix Pharmaceuticals, USA) that were combined with rabbit antisera against calbindin D-28K (1:2000; code CB-38a, Swant, Switzerland) or mouse monoclonal antisera against either calretinin (1:2000; code 6B3, Swant, Switzerland) or parvalbumin (1:2000, code P3088, Sigma–Aldrich, USA). The specificity of both antibodies against CART was assessed in our previous studies (Równiak et al., 2010; Bogus-Nowakowska et al., 2011, 2012). In order to visualize the binding sites of the antigens–antisera used, sections were then incubated (1 h, at room temperature) with a mixture of FITC-conjugated donkey anti-mouse (1:400, code 715-095-150, Jackson ImmunoLabs, USA) or FITC-conjugated donkey anti-rabbit antibody (1:400, code 711-095-152, Jackson ImmunoLabs, USA) combined with either Cy3-conjugated donkey anti-rabbit (1:8000; code 711-165-152, Jackson ImmunoLabs, USA) or Cy3-conjugated donkey anti-mouse antibody (1:8000; code 715-165-150, Jackson ImmunoLabs, USA). All antibodies were diluted in PBS containing Triton X-100 (0.3–0.5%) and 1% normal donkey serum. Following antibody incubations, sections were then washed 3 times in 0.1 M PBS and were coverslipped in buffered carboxyglycerol (pH 7.8). The omission of the primary antibody served as a negative control.

Data analysis

Sections were analyzed using an Olympus B X51 microscope equipped with a CCD camera connected to a computer. Images were acquired and cell measurements were made with the Cell F software (Olympus GmbH, GER). The length of the immunostained perikarya was determined by measuring the major axis at 400× magnification. Cell measurements were expressed as mean value ± standard error of the mean (SEM).

Results

Subdivisions of the guinea pig medial geniculate body

In coronal sections of Nissl preparations, the MGB of the guinea pig forms a knee shaped protuberance on the lateral edge of the diencephalon. In the rostral part, it is dorsally bordered by the lateral geniculate body (Fig. 1a), which is replaced by the superior colliculus in the medial and caudal part (Fig. 1b and c). Ventrally the MGB is bordered by the substantia nigra along most of its rostro-caudal extent (Fig. 1a–c). Similarly, as in other species, the guinea pig MGB consists of three major cytoarchitectural areas: the MGd, MGv and MGm. All three divisions were present throughout the entire length of the MGB, except the short, initial segment of the rostral part, which was comprised entirely of the MGv. Among the three primary divisions of the MGB, the MGv is the largest in size with a rounded shape (Fig. 1a–c). The MGv contained most densely packed, medium sized cells (Fig. 2c and d). The MGd is ovoid shape, but smaller in size (Fig. 1a–c) and containing slightly smaller and more scattered nerve cells (Fig. 2a and b) than the MGv. The MGm was the smallest, irregulary shaped division (Fig. 1a–c), characterized by the most various neurons and the lowest cell density (Fig. 2e and f). In this study all three parts of the MGB were studied to describe the distribution of CART and three calcium binding proteins (calbindin, calretinin and parvalbumin).