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Cocaine- and amphetamine–regulated transcript peptide and calcium binding proteins immunoreactivity in the superficial layers of the superior colliculus in the guinea pig: Implications for visual sensory processing

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ABSTRACT

The purpose of this study was to investigate the distribution and colocalization of cocaine- and amphetamine-regulated transcript peptide (CART) and three calcium-binding proteins (calbindin, calretinin and parvalbumin) in the superficial layers of the superior colliculus (SCs) in the guinea pig. The CART immunoreactivity was observed exclusively in the solitary fibers and neuropil, which formed various CART-ir tiers, that corresponded partially or entirely to anatomically defined layers of the SCs. The CART-ir structures exhibited a characteristic morphology with bundles of densely intermingled neuronal fibers and terminals. This pattern of CART immunoreactivity in the visually driven SCs strongly indicates that CART peptide as a putative neurotransmitter may play an important role in processing of visual information. Double-labeling immunofluorescence showed that CART did not colocalize with either calcium binding proteins (CaBPs). Immunolabeling for CaBPs revealed the presence of different neuronal populations, which were concentrated in variously pronounced tiers. Contrary to CART, the CaBPs immunoreactivity in perikarya was relatively high and CaBPs containing neurons displayed a variety of sizes and somatodendritic morphologies. Generally, CaBPs patterns in the SCs of the guinea pig differ, to some extent, from those of other rodents. These results prove the importance of studying the neurochemical cytoarchitecture of diverse mammals.

1. Introduction

Cocaine- and amphetamine-regulated transcript peptide (CART) is a putative neurotransmitter, which was found to be expressed throughout the central and peripheral nervous systems (Koylu et al., 1998; Jaworski and Jones, 2006; Janzsó et al., 2010; Równiak et al., 2010; Żakowski et al., 2014; Wasilewska et al., 2016). Additionally, CART was found in endocrine cells in the pituitary and adrenal glands (Koylu et al., 1997). In the brain, the distribution of this relatively novel neuropeptide seems to be wide, but selective. As a result, the spectrum of its activity is also considered to be very wide, such as the regulation of food intake, reward and reinforcement related behaviors, drug abuse, reproductive and maternal behaviors, and sensory information processing (Hurd and Fagergren 2000; Jaworski and Jones, 2006; Janzsó et al., 2010; Bogus-Nowakowska et al., 2011, 2012). The few studies that have examined the localization of CART in the sensory related brain regions have shown that this peptide is present in the olfactory bulb, primary olfactory cortical areas and in the retina (Couceyro et al., 1997; Koylu et al., 1998). Our recent report (Najdzion et al., 2014) indicates that CART could be involved in the hearing process, although its immunoreactivity in the medial geniculate body (MGB) was not as intense as in other sensory brain regions. The superficial layers of the superior colliculus (SCs) are concerned exclusively with the processing of visual information (Lund et al., 1980; Petry et al., 1989; Harvey and Worthington, 1990; Sugita et al., 1983; Lugo-Garcia and Kicliter, 1987; Harting et al.,

Abbreviations: CaBPs, calcium binding proteins; CART, cocaine- and amphetamine-regulated transcript peptide; CB, calbindin; CR, calretinin; IGL, intergeniculate leaflet; LFB, luxol fast blue; LGNd, dorsal nucleus of the lateral geniculate body; LGNv, ventral nucleus of the lateral geniculate body; MGB, medial geniculate body; PUL/LP, pulvinar/lateral posterior complex; PV, parvalbumin; SC, superior colliculus; SCs, superficial layers of the superior colliculus; SGS, stratum griseum superficiale; SO, stratum opticum; SZ, stratum zonale.

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1991). The fact of presence of CART in the mammalian SCs is known, but there is not yet detailed information regarding CART distribution in this brain region. Therefore, the purpose of the present study was to provide a comprehensive morphological description of CART-ir structures and their spatial locations in the SCs of the guinea pig, a species that has some anatomical and functional features of sensory systems different from other rodents. For instance, the guinea pig differently from the rat and mouse, has a paurangiotic retina (Rodriguez-Ramos Fernandez and Dubielzig, 2013) and both A- and B-type retinal horizontal cells (Peichl and González-Soriano, 1994). Furthermore, it has a large tympanic bullae with easily accessible internal ear and hearing range that is similar to that of humans. For these reasons, the guinea pig has become a popular model organism for investigations of sensory processing (Lossi et al., 2016). Consequently, the distribution pattern of CART in the SCs of this species could shed some light on a potential role of CART in visual processing.

The calcium binding proteins (CaBPs) belong to the large EF-hand family. Even though they abundantly occur in different types of neurons in the central nervous system, the precise functions of these proteins are not completely clear. Besides their proven calcium buffer function, they may actively work in calcium-mediated signal transduction (Schwaller, 2009). This potential "new" function" for "old" proteins is a reason that the role of CaBPs in brain physiology has been widely studied. Although neurons expressing CaBPs have been described in the SCs of different mammals, a comprehensive information on CaBPs immunoreactivity in the SCs of the guinea pig has not been yet reported. It is particularly worth investigating, because there have been studies showing that CaBPs distribution may vary considerably amongst species (Pasteels et al., 1990; McHaffie et al., 2001). Therefore, the information about the distribution of CaBPs and interspecies differences contributes an important framework for physiologists and could be very useful for studying the visual system. Moreover, CaBPs are usually restricted to specific types of neurons and rarely colocalize with each other. They serve as useful functional markers for neurons that are assumed to have some unifying physiological properties. Thus, there have been many attempts to find a correlation between the distribution of CaBPs and various neurochemical substances, including CART. It was reported that CART along with calbindin (CB) might be involved in the development of the hippocampus, and both these neuropetides might play a neuromodulatory role in neurons of the dentate gyrus (Abrahám et al., 2007). The study by Żakowski et al. (2014) demonstrated the coexistence pattern of CB and CART in the mammilar body-anterior thalamic nuclei axis of the guinea pig, which suggested the possible involvement of these neuropeptides in mnemonic processes. Contrarily, no colocalization was detected between CART and CaBPs in the guinea pig subicular complex, which is considered to constitute the major output station of the hippocampal formation, and consequently contribute to learning and memory, as well as other cognitive functions (Wasilewska et al., 2016). These results indicate that colocalization between CART and CaBPs might be specific only to certain parts of the functional pathways of the brain. Our previous study (Najdzion et al., 2014) showed that CaBPs do not colocalize with CART in the MGB, which is an essential part of the auditory pathway. Since the SCs are exclusively connected with the structures of the visual system, I hypothesized that the colocalization pattern in the SCs might be different from that in the MGB. Therefore, I also aimed to determine whether or not CART colocalizes with CB, calretinin (CR) and parvalbumin (PV).

2. Material and methods

2.1. Animals and tissue processing

The study was performed on 6 sexually mature female guinea pigs (strain: Dunkin-Hartley, average weight 600 g, obtained from the Research Institute of the Polish Mothers' Health Centre in Łódź, 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 anaesthetized 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.

2.2. 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–28 K (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 sections were kept for 15 h, at room temperature. In order to visualize the binding sites of the antigens-antisera used, the 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.

2.3. Data analysis

Sections were analyzed using an Olympus B X51 microscope equipped with a CCD camera connected to a computer. Images and cell measurements were acquired with the Cell F software (Olympus GmbH, GER). In order to avoid bias all images were analyzed and captured under the same settings. When necessary, the final images were adjusted for sharpness, brightness and contrast using GIMP (version 2.8.16) to reflect the appearance of the labeling, seen under the microscope. In order to maintain the real appearance of the tissue, no blemishes on the sections were removed from the final images. The length of the immunostained perikarya was determined by measuring the major axis at 400× magnification. Cell measurements were expressed as mean value ± standard deviation (SD).