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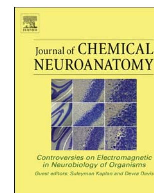

Cocaine- and amphetamine–regulated transcript peptide and calcium binding proteins immunoreactivity in the deep layers of the superior colliculus of the guinea pig: Implications for multisensory and visuomotor processing

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ABSTRACT

The superior colliculus (SC) of mammals is a midbrain center, that can be subdivided into the superficial (SCs) and deep layers (SCd). In contrast to the visual SCs, the SCd are involved in multisensory and motor processing. This study investigated the pattern of distribution and colocalization of cocaine- and amphetamine-regulated transcript peptide (CART) and three calcium-binding proteins (CaBPs) i.e. calbindin (CB), calretinin (CR) and parvalbumin (PV) in the SCd of the guinea pig. CART labeling was seen almost exclusively in the neuropil and fibers, which differed in regard to morphology and location. CART-positive neurons were very rare and restricted to a narrow area of the SCd. The most intense CART immunoreactivity was observed in the most dorsally located sublayer of the SCd, which is anatomically and functionally connected with the SCs. CART immunoreactivity in the remaining SCd was less intensive, but still relatively high. This characteristic pattern of immunoreactivity indicates that CART as a putative neurotransmitter or neuromodulator may play an important role in processing of visual information, while its involvement in the auditory and visuomotor processing is less significant, but still possible. CaBPs-positive neurons were morphologically diverse and widely distributed throughout all SCd. From studied CaBPs, CR showed a markedly different distribution compared to CB and PV. Overall, the patterns of distribution of CB and PV were similar in the entire SCd. Consequently, the complementarity of these patterns in the guinea pig was very weak. Double immunostaining revealed that CART did not colocalize with either CaBPs, which suggested that these neurochemical substances might not coexist in the multisensory and visuomotor parts of the SC.

1. Introduction

The superior colliculus (SC) of mammals is a midbrain center of visual-motor integration, which can be anatomically and functionally subdivided into the superficial (SCs) and deep layers (SCd). Traditionally these layers have been considered as separate structures (Sprague, 1975), although there are strong connections and interactions between them. The SCs are exclusively visual (Lugo-Garcia and Kicliter, 1987; Harvey and Worthington, 1990; Harting et al., 1991), while the SCd are involved in multisensory and motor processing, and their neurons respond to combinations of visual, auditory and somatosensory stimuli. Consequently, the SCd receive broad projections from various cortical and subcortical areas and then transmit signals to the midbrain and pontine gaze centers, that organize and initiate saccadic eye movements (Edwards et al., 1979; Cowie and Holstege, 1992; Harting et al., 1992; May and Porter, 1992).

Cocaine- and amphetamine-regulated transcript mRNA was discovered in the rat striatum after acute administration of cocaine or amphetamine (Douglass et al., 1995). Lately, it turned out that C-terminus of the peptide encoded by this mRNA was identical to a peptide fragment of unknown function, which had been previously isolated by Spiess et al. (1981) from the ovine hypothalamus. Since then, several subsequent studies have demonstrated that cocaine- and amphetamine-regulated peptide (CART) could serve as a putative neurotransmitter or neuromodulator, which is distributed throughout the central and peripheral nervous systems (Koylu et al., 1998; Jaworski and Jones, 2006;

Abbreviations: CaBPs, calcium binding proteins; CART, cocaine- and amphetamine-regulated transcript peptide; CB, calbindin; CR, calretinin; LFB, luxol fast blue; MGB, medial geniculate body; MGm, medial division of the medial geniculate body; PAG, periaqueductal gray; PV, parvalbumin; SAI, stratum album intermediale; SAP, stratum album profundum; SC, superior colliculus; SCd, deep layers of the superior colliculus; SCs, superficial layers of the superior colliculus; SGI, stratum griseum intermediale; SGP, stratum griseum profundum; SO, stratum opticum

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Janzsó et al., 2010; Równiak et al., 2010; Gonkowski et al., 2013; Wasilewska et al., 2016; Zacharko-Siembida et al., 2017). Currently, CART is believed to be involved in various activities, including the regulation of food intake, reward and reinforcement related behaviors, drug abuse, reproductive and maternal behaviors, and sensory information processing (Couceyro et al., 1997; Koylu et al., 1998; Hurd and Fagergren, 2000; Jaworski and Jones, 2006; Janzsó et al., 2010; Bogus-Nowakowska et al., 2011, 2012; Najdzion et al., 2014). My recent findings (Najdzion, 2017) showed that CART was abundant in the SCs, that strongly suggested its potential role in processing of visual information. Since the SCd are concerned with the multisensory integration, I hypothesized that the distribution pattern of CART in the SCd might be different from that in the visually driven SCs. Therefore, the purpose of this study was to provide the first comprehensive morphological description of CART-ir structures and their spatial locations in the SCd of the guinea pig, a species that has become a popular model organism for investigations of sensory processing.

The calcium binding proteins (CaBPs) are common in the nervous system, but their functions are not completely clear. Currently, CaBPs are being considered as calcium buffers, however, recent results indicate that they might have additional Ca(2+) sensor functions (Schwaller, 2009). Although, there were studies reporting the presence of CaBPs in the mammalian SCd, a comprehensive information on CaBPs immunoreactivity in the SCd of the guinea pig has not been yet reported. Moreover, there are inconsistent results regarding the distribution of calretinin (CR) in the SCd of rodents. For the purposes of the present study, it is also important to note that CaBPs could serve as useful markers of different neuronal populations, since they are usually restricted to specific types of neurons that are assumed to have some unifying physiological properties. Furthermore, CaBPs rarely colocalize with each other, while they often coexist with different neurochemical substances in neurons. There have been reports showing that the colocalization between CART and CaBPs might be specific only to certain brain regions that participate in cognitive functions (Abrahám et al., 2007; Żakowski et al., 2014; Wasilewska et al., 2016). My previous studies (Najdzion et al., 2014; Najdzion, 2017) indicated that CART did not coexist with calbindin (CB), CR and parvalbumin (PV) in some cerebral structures of the visual and auditory pathways. Thus, in the present study I aimed to determine whether or not CART colocalizes with these CaBPs in the multisensory part of the brain. These results together with earlier work (Najdzion, 2017) will constitute a complete characterization of CART, CB, CR and PV immunoreactivity in the SC of the guinea pig, and will contribute to our understanding of the organization and functions of this multilayered structure. Furthermore, this will facilitate comparison with existing data from other species, which will provide a better understanding of the parallels and differences among species from different orders.

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. Animal procedures followed the guidelines established by the Animal Care and Use Ethical Committee of the University of Warmia and Mazury in Olsztyn. 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 body weight) and perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4) with 1N NaOH added. 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 graded solutions (19% and 30%) of sucrose (Sigma Aldrich) at 4 °C until they sunk. 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-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 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).

2.3. Controls

In the preliminary studies, the primary antibodies that were produced in different species (mouse and rabbit anti-CB, -PV, -CR, and -CART) and provided by different manufacturers (Sigma, Swant, Phoenix Pharmaceuticals, R&D) were tested on the guinea pig tissue. The staining patterns were identical for all variants of the antibodies used, and therefore proved that their specificities were similar. Moreover, the specificity of some primary antisera has been tested by the manufacturers. For example, product descriptions of rabbit anti-calbindin (Swant, CB-38a) and mouse anti-calretinin (Swant, 6B3) antisera include immunoblots of the guinea pig brain homogenates, which were specifically stained by these antibodies, showing bands at 28 kDa and 29 kDa, respectively. The same primary antibodies were applied in our laboratory in previous studies (Bogus-Nowakowska et al., 2011, 2012; Najdzion et al., 2014; Żakowski et al., 2014; Wasilewska et al., 2016; Najdzion, 2017) as well as by other authors (Solbach and Celio, 1991; Airaksinen et al., 1997; Zimmermann and Schwaller, 2002; Ellis and Mawe, 2003; Hunter et al., 2005; Mészár et al., 2012). To test the specificity of the secondary antibodies, the omission and/or replacement of all primary antisera by non-immune sera was performed. A lack of immunoreactions indicated specificity.

2.4. Luxol fast blue staining

Tissue sections were stained in Luxol Fast Blue solution (0.1% Luxol Fast Blue MBS in 95% ethanol with 0.5% acetic acid) at 56 °C overnight. The sections were rinsed in 95% ethanol and in distilled water, dipped briefly (3 to 5 s) in lithium carbonate solution (0.05%), differentiated (20 to 30 s) in 70% ethanol, and then washed in distilled water. Next, the sections were counterstained in cresyl violet solution (0.1% cresyl fast violet in distilled water with 10 drops of glacial acetic acid) for 30–40 s, rinsed in distilled water, differentiated in 95% ethanol for 5 min, and then dehydrated in absolute ethanol (2 × 5 min), cleared in xylene (2 × 5 min), and mounted in Canada balsam for microscopy.

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