Cocaine- and amphetamine-regulated transcript (CART) peptide in spinal neuronal networks involved in nociceptive information processing

Ph.D. thesis – Summary

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Cocaine- and amphetamine-regulated transcript (CART) peptide has been implicated in regulation of several physiological functions including reward, food-intake and neuroendocrine functions. The role of CART peptide in spinal nociception has been suggested by behavioural studies and dense plexus of CART-immunoreactive fibres has been described in the superficial laminae of the spinal cord, which are key areas of the pain transmission, but the anatomical evidence for the involvement of CART peptide in the spinal nociception has been completely missing.

We used antibody against CART peptide, together with markers for various types of primary afferents, interneurons and descending systems to determine the origin of the CART-immunoreactive axons in the superficial laminae of the rat spinal cord. Calcitonin gene-related peptide (CGRP), a marker for peptidergic primary afferents in the dorsal horn, was present in 72.6% and 34.8% of CART-immunoreactive axons in lamina I and II, respectively. The majority of these fibres also contained substance P (SP). The other subpopulation of CART-immunoreactive boutons in laminae I-II also expressed SP and/or somatostatin (SOM) without CGRP, but contained vesicular glutamate transporter 2, which is present mainly in excitatory interneuronal terminals. In lamina I, CART peptide was present in half of the CGRP-immunoreactive terminals and many of the CART/CGRP-ergic boutons were also immunoreactive for galanin (GAL), which is one of the key peptides in chronic pain states. Electron microscopy showed that most of the CART terminals formed asymmetrical synapses mainly with dendrites. Using retrograde tract tracing and multiple immunofluorescent labelling, the relationship between CART and/or CGRP axons and projection neurons, playing a pivotal role in forwarding painful stimuli toward supraspinal levels, were examined. The contact density analysis of CART-ergic and/or CGRP-containing fibres terminating on lamina I spinoparabrachial projection neurons showed that all examined cells received contacts, but the innervation density did not differ significantly between either of the different neurochemical or the morphological subclasses of these cells.

Our data demonstrate that the majority of CART-immunoreactive axons in the spinal dorsal horn originate from peptidergic nociceptive primary afferents, while a smaller proportion of them arises from local interneurons. The co-existence of CART with nociceptive peptides in primary afferents and excitatory interneurons suggests that the peptide can affect glutamatergic neurotransmission as well as the release and effects of SP, SOM and GAL in nociception and other sensory processes. Furthermore, we revealed a direct non-selective input from CART-immunoreactive axons to lamina I projection neurons.

These results provide anatomical bases for involvement of CART peptide in spinal pain transmission.

1. Kozsurek M, Lukácsi E, Fekete Cs, Wittmann G, Réthelyi M, Puskár Z. (2007) Cocaine- and amphetamine-regulated transcript peptide (CART) is present in peptidergic C primary afferents and axons of excitatory interneurons with a possible role in nociception in the superficial laminae of the rat spinal cord. *Eur J Neurosci*, 26 (6), 1624-1631.

2. Kozsurek M, Lukácsi E, Fekete Cs, Puskár Z. (2009) Nonselective innervation of lamina I projection neurons by cocaine- and amphetamine-regulated transcript peptide (CART)-immunoreactive fibres in the rat spinal dorsal horn. *Eur J Neurosci*, 29:2375-2387.