Commentary: GPR160 De-Orphanization Reveals Critical Roles in Neuropathic Pain in Rodents (Finally, a Receptor for CART Peptide)

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A Commentary on

GPR160 de-orphanization reveals critical roles in neuropathic pain in rodents

by Yosten GLC, Harada CM, Haddock C, Giancotti LA, Kolar GR, Patel R, Gou C, Chen Z, Zhang J, Doyle TM, Dickenson AH, Samson WK, and Salvemini D. (2020). *J Clin Invest.* 130:2587–92. doi: 10.1172/JCI133270

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Signaling in rat brainstem via Gpr160 is required for the anorexigenic and antidipsogenic actions of cocaine- and amphetamine-regulated transcript peptide

by Haddock CJ, Almeida-Pereira G, Stein LM, Hayes MR, Kolar GR, Samson WK, et al. (2021). *Am J Physiol Regul Integr Comp Physiol.* 320:R236–49. doi: 10.1152/ajpregu.00096.2020

This commentary focuses on recent publications identifying a likely Cocaine-and-Amphetamine Regulated Transcript (CART) peptide (CARTp) receptor, namely the recently de-orphanized GPR160, some 26 years after the discovery of the CART mRNA. These publications are Yosten et al. [1], and Haddock et al. [2].

BACKGROUND

The discovery of the CART transcript and peptides implicated CARTp as a neurotransmitter involved in the action of psychostimulants and neuroendocrine regulation [3, 4]. But it became evident that it was involved in many additional physiological processes including body weight and feeding, energy expenditure, physical activity, body temperature, endocrine regulation, drug abuse and reward, pain, stress, hypertension, anxiety and depression, recovery from stroke, and possibly others. There are many reviews and other citations describing the evidence for these effects [5–30]. The general belief was and is that CARTp is an important peptide neurotransmitter. Several peptides derived from proCART are likely active [10, 14, 31].

Following the exciting discoveries of the peptide's involvement in drug abuse [4, 7, 10, 11, 17, 30] and other processes, a critical need was for the identification and cloning of a CARTp receptor. There was evidence that a receptor existed, and it is briefly as follows. Responses to injections of CARTp were dose-responsive and the active peptide had structural requirements [14, 18, 22, 27, 30–40]. Injections of CARTp increased levels of second messengers, a common post receptor event [20, 21,

25, 28, 41–43]. Radiolabeled CARTp showed displaceable binding to neuronal cultured cells membranes, although and unusually so, not to adult brain tissue membranes [20, 27, 32, 42]. CARTp binding was altered by Gpp (nh)p - a G-protein binding ligand, and CARTp enhanced the binding of (35)S-GTP gamma S; these and other studies suggested that the receptor was a GPCR coupled to Gi/o [19–21, 28, 43, 44]. See also the reviews cited at the end of the last paragraph.

RECENT DISCOVERIES

Given the existing evidence that a CARTp receptor is a GPCR, a reasonable approach to searching for a CART peptide receptor is to examine GPCR orphan receptors, receptors for which there are no known neurotransmitter ligands [29]. In studies of neuropathic pain, Yosten et al [1] found that an orphan receptor, GPR160 played a significant role in neuropathic pain and spinal cord. GPR160 was increased in spinal cord after traumatic nerve injury. Also, inhibition of GPR160 in the spinal cord prevented and reversed neuropathic pain but had no effect on normal pain. They then examined the connection between CARTp and GPR160 using antibodies (ab) and short interfering RNAs (si).

In KATO cancer cells, CARTp-induced cFOS expression and this was blocked by prior depletion of GPR160 using an si-GPR160. In PC12 cells expressing GPR160, CARTp stimulated ERK phosphorylation, but prior treatment with si-GPR160 reduced the effect. Also, CARTp co-immunoprecipitated with GPR160 protein indicating the likelihood of a physical interaction *in vivo*. Another finding was that injection of CARTab mimicked the effects of GPR160 inhibition. A CARTp-induced mechanohypersensitivity was dependent on GPR160. CARTp induced phosphorylation of ERK but this was attenuated with coinjection of GRP160ab. These data showed a functional and physical connection between CARTp and GPR160, at least in neuropathic pain.

Haddock et al [2] studied the CARTp receptor problem in the context of food and water intake. Injection of CARTp into the fourth ventricle reduced food and water intake, and this was prevented by immuno-neutralization of GPR160, again a connection between CARTp and GPR160. A hypothesis of circuitry and cellular localizations was made to provide a possible mechanism for these observations.

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DISCUSSION

These findings strongly link the effects of CARTp to binding to an orphan receptor, GPR160. This initial identification of a CARTp receptor is a welcome observation after so many years of searching. Additional studies and confirmation of these findings are needed.

Could there be other CARTp receptors? It seems likely since most neurotransmitters have multiple receptors, and as noted above and elsewhere [45], there are several slightly different CARTps in different species and organs. In the recent studies discussed here, CARTp and GPR160 are implicated in neuropathic pain and food and water intake. Other receptors may be involved with other functions of CARTps.

This discovery will facilitate further research and understanding of the CARTp system in brain and periphery. It will also facilitate screening for small molecule agonists and antagonists, of which some mention has been made [20]. This will be very helpful in further studies of the functions of CARTps and for identifying therapeutic compounds based on CART.

AUTHOR CONTRIBUTIONS

Both MJ and MK contributed equally to the article. MK wrote the original draft of the article. MJ prepared the final draft of the article for submission.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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