

Relationships of Cocaine and Amphetamine Regulated Transcript with Serotonin in the Brain

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Cocaine and amphetamine-regulated transcript (CART) is a satiety factor that is regulated by leptin. It was reported that the mice intracerebroventricularly injected with CART showed behavioral changes resembled with the typical behavioral alterations found in the mice carrying disorders in the brain serotonergic (5-HT) system. Hence, this study was conducted to find out the relationships between CART and 5-HT. We first examined the mRNA levels of CART after the injections of para-chlorophenylalanine (pCPA, 300 mg/kg i.p., single injection or daily for three consecutive days) in the rat brains by *in situ* hybridization using the mouse CART cDNA probe cloned in our laboratory. Systemic administrations of pCPA, a potent inhibitor of tryptophan hydroxylase, the rate limiting enzyme of 5-HT biosynthesis, acutely depletes the brain 5-HT transporter (5-HTT) in the dorsal raphe nucleus (DRN), which reuptakes terminal 5-HT. Results indicated that the mRNA level of CART significantly decreased in the arcuate nucleus, paraventricular nucleus, and lateral hypothalamic nucleus by three days of daily injection with pCPA with no noticeable change detected 24 hrs after the single injection. The message levels of 5-HTT in DRN decreased in both single and three days of injections. Secondly, to investigate whether CART affect to 5-HT, mouse genomic CART gene, which is consist of 3 exons and 2 introns and mouse neurofilament light (NF-L) chain promoter were cloned. Then, we constructed neuron specific expression vector, which was transfected into HeLa cell using lipid-mediated transfection system. Expression of GFP and CART linked to NF-L-chain promoter in the transfected HeLa cell were detected by using fluorescent microscope and RT-PCR. These results confirmed normal expression of DNA constructs *in vitro*. Then, to increase brain specific expression of CART *in vivo*, transgenic mice carrying CART gene controlled the deleted NF-L-chain promoter were generated by the DNA microinjection into pronuclei of fertilized embryos. Transgenic mice were detected by Southern blot. Further study is necessary to examine CART expression and 5-HTT in these transgenic mice. Therefore, these results suggest that there maybe a positive molecular correlation between CART and 5-HT in responding to the stimuli.

(Key words) *CART, 5-HT, In situ hybridization, NF-L-chain promoter, Transgenic mice*