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New unique analogs of anorexigenic cocaine- and amphetamine-regulated transcript peptide

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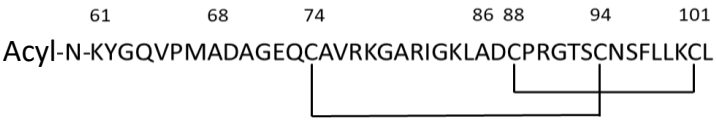
Scientific background: Cocaine- and amphetamine-regulated transcript peptide (CARTp) is an important anorexigenic neuropeptide expressed in the hypothalamus that contains three disulfide bridges important for the biological activity [1]. Its expression is stimulated by leptin, the main regulator of energy homeostasis, which highlights its importance in food intake regulation [2]. CARTp receptor is still unknown, however, Maletínská et al. described the specific binding of CARTp to pheochromocytoma PC12 cells [3]. Subsequent study of Blechová et al. defined the CARTp analog CART(61-102) with only two disulfide bridges, 2-SS-CART(61-102), as the shortest analog with preserved binding affinity and food intake-lowering properties after application to the 3rd brain ventricle [4]. However, natural CARTp is not capable of central action after peripheral administration. Therefore, we designed new lipidized analogs that were tested for their potential anorexigenic properties.

Methods: Lipidized analogs of 2-SS-CART(61-102) were designed and synthesized at the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences in Prague (IOCB CAS in Prague) using Fmoc strategy. The purity and identity of all lipidized analogs were determined by analytical HPLC and using a MALDI-TOF/TOF (Bruker Daltonics, Germany)/Q-TOF micro (Waters) MS technique. The structures are shown in Table 1.

Table 1 Structure of 2-SS-CART(61-102) and its lipidized analogs

Analogue	Sequence
2-SS-CART(61-102)	
acyl-2-SS-CART(61-102)	

Acyl: octanoyl (oct), mirystoyl (myr), palmitoyl (palm)

Their binding affinity was tested in PC12 cells, either in naïve cells or those differentiated to neuronal phenotype by nerve growth factor (NGF). The effect on food intake regulation and body weight change was investigate after chronic subcutaneous (SC) application to mice with obesity induced by application of monosodium glutamate (MSG) to newborn mice, day 2-5 after birth, at a dose 4 mg/kg.

Results and discussion: All lipidized analogs showed binding affinity to PC12 cells, naïve or NGF-differentiated, comparable to 2-SS-CART(61-102), as shown in Table 2. palm-2-SS-CART(61-102) showed even higher binding affinity than the natural CART(61-102) with three disulfide bridges [3].

Table 2 Binding affinity of lipidized 2-SS-CART(61-102) analogs

Analog	Naïve PC12 cells	NGF-differentiated PC12 cells
	^{125}I-CART(61–102) K_i [nM]	^{125}I-CART(61–102) K_i [nM]
2-SS-CART(61–102)	261.3 ± 209,80	10.26 ± 9.04
oct-2-SS-CART(61–102)	76.09 ± 17.66	43.9 ± 10.41
myr-2-SS-CART(61–102)	12.1 ± 3.40	14.34 ± 0.33
palm-2-SS-CART(61–102)	1.43 ± 0.41	0.39 ± 0.14

The palm-2-SS-CART(61-102) was subsequently SC administered for 3 weeks to MSG obese mice. The treatment significantly reduced food intake and body weight of the MSG mice. Furthermore, palm-2-SS-CART(61-102) decreased the plasma level of leptin, which is produced by white adipose tissue, and insulin. It is possible that palm-2-SS-CART(61-102) regulates the activity of pancreatic β-cells [5]. In conclusion, we designed new unique CARTp analogs with central effect after peripheral application with strong anorexigenic and antiobesity potential.

Dedication:

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