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European Journal of Pharmacology 528 (2005) 188–189
www.elsevier.com/locate/ejphar

Rapid communication

CART (cocaine- and amphetamine-regulated transcript) peptide receptors: Specific binding in AtT20 cells

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Received 23 November 2005; accepted 28 November 2005

Abstract

Given previous evidence for CART (cocaine- and amphetamine-regulated transcript) signaling in AtT20 cells, the binding of [^{125}I]-CART61–102 was characterized in these cells. The binding was specific, saturable, dependent on time, pH, temperature and protein concentration, with a B_{max} of 101.4±8.8 fmol/mg protein and a K_d of 21.9±8.0 pM. Only active CART55–102, but not other peptides or drugs, inhibited the [^{125}I]-CART61–102 binding. These data are the first demonstration of specific receptor binding for CART peptides.

Keywords: Feeding; Obesity; Drug abuse

CART peptides are neurotransmitters involved in feeding and body weight, drug abuse, stress and neuroendocrine control (Kuhar et al., 2005; Murphy, 2005). Despite the clear evidence for behavioral and cellular activity of the peptides, the receptors for CART peptides have not yet been found in spite of years of searching. Recently, we found that CART55–102 activates extracellular regulated kinase (ERK) signaling in AtT20 cells via a G-protein-coupled receptor (Lakatos et al., 2005). Accordingly, the present study utilized AtT20 cells to identify specific CART peptide receptor binding.

AtT20 cells were grown as previously described (Lakatos et al., 2005). The binding buffer consisted of 20 mM HEPES, pH 6.5, 5 mM $MgCl_2$, 150 mM NaCl, 1 mM EGTA, and included a protease inhibitor cocktail with 100 μM 4-(2-aminoethyl) benzenesulfonyl fluoride, 70 μM E-64, 65 μM bestatin, 50 μM leupeptin, 15 μM aprotinin, and 50 μM EDTA. The incubations with [^{125}I]-CART61–102 (1546 Ci/mmol; Phoenix Pharmaceuticals, CA) were carried out with cells attached to plates for 3 h on ice with 10 μM CART55–102 added to define non-specific binding. Cells were washed 2×1 min with fresh buffer and solubilized. 30 pM [^{125}I]-CART61–102 was used for competition experiments.

Total [^{125}I]-CART61–102 binding was mostly displaced by CART55–102 and this specific binding was linear with protein concentrations up to about 150 μg/ml. The specific binding increased with time to reach a plateau at about 3 h and remained stable for at least an additional 90 min. The saturable, specific [^{125}I]-CART61–102 binding exhibited an average K_d of 21.9±8.0 pM and B_{max} of 101.4±8.8 fmol/mg protein determined by nonlinear analysis (Fig. 1). Scatchard analysis demonstrated similar high affinity [^{125}I]-CART61–102 binding to an apparent single population of sites with a K_d of 39.1 pM and a B_{max} of 90.43 fmol/mg protein (Fig. 1, inset). The specific binding was eliminated by preincubation of the cells at 100 C for 15 min, and was selective since only active CART55–102 peptide (Peptides International, KY) displaced it with a K_i value of 17.1±4.2 pM. The inactive CART fragments (CART 57–79 and CART 28–54), angiotensin II, vasopressin (Phoenix Pharmaceuticals, CA), NPY, glucagon-like peptide and GABA (Sigma Chemicals), cocaine, or Gallus peptide (PTP GED DDI PVK C; Gallus Immunotech, NC) did not displace the binding, even at 10 μM concentrations (not shown). All assays were performed in triplicate six times.

ProCART consists of two forms in the rat, 89 or 102 amino acids (Douglass et al., 1995) while in the human the 89 amino acid form predominates. Processing of proCART yields several fragments in the rat including the active fragments 55–102 and

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doi:10.1016/j.ejphar.2005.11.041

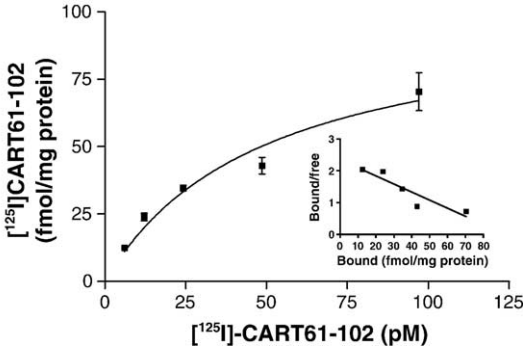

Fig. 1. Saturation binding of [^{125}I]-CART61–102 and (inset) Scatchard plot. AtT20 cells were incubated with increasing concentrations (6 pM–0.1 nM) of [^{125}I]-CART61–102. 10 μM CART55–102 was added to define non-specific binding. The figure shows a representative saturation curve. The data points represent mean+S.E.M (n=3).

62–102. CART 61–102 has agonist properties (not shown), and similar to 55–102, stimulates phosphorylation of ERK in AtT20 cells (Lakatos et al., 2005). The results of this study demonstrate that an apparent CART receptor exists in AtT20 cells as we provide evidence of specific binding of [^{125}I]-CART61–102.

In summary, this is the first evidence for specific CART peptide receptor binding. This notable success is likely due to selecting a tissue that exhibits CART 55–102 signaling (Lakatos et al., 2005) and to identifying a buffer and incubation conditions that were successful. The presence of the receptor in brain was suggested in earlier studies demonstrating that CART peptide is behaviorally active, inhibits voltage-dependent intracellular Ca^{2+} signaling (Yermolaieva et al., 2001) and induces c-*fos* expression (Vrang et al., 1999). Moreover, there might be multiple CART receptors as CART 55–102 and CART 62–102 differ in their relative potencies in various tests (Bannon et al., 2001; Thim et al., 1998). In any case, future experiments should further characterize putative CART receptor binding and facilitate the cloning of a CART receptor cDNA. Given the role of CART in feeding, stress, psychostimulant drug action and development, the identification of the CART receptor will facilitate the search for molecules that could be employed as therapeutic agents to mimic or block the endogenous actions of CART peptides.

Acknowledgements

The authors thank Ms. Lili Shen, Dr. Ken Minneman and Dr. Randy Hall and the work was supported by grants RR00165, DA10732 and DA00418.

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