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Targeting Trends

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The Biologically Active Cholecystokinin (26-33) peptide, [Tyr^2-SO$_3$]CCK-8, Retains High Affinity for CCK$_2$ Receptors after Covalent Conjugation to Saporin

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Cholecystokinin (CCK) is widely distributed in the central nervous system and the gastrointestinal tract. The 33-amino acid peptide contains a carboxyl terminal octapeptide sequence Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH$_2$ which confers the biological activity of CCK, and where the tyrosine residue occurs in sulfated form. This octapeptide, CCK-8(SO$_3$), has high affinity for the two structurally-defined CCK receptor types, CCK$_1$ and CCK$_2$. A covalent conjugate of CCK-8(SO$_3$) to saporin (CCK-8(SO$_3$)-SAP) was synthesized and evaluated for the toxin conjugate's affinity for the human CCK$_2$ receptors in transfected HEK293 cells (Figure 1). The K_i value of CCK-8(SO$_3$) for the CCK$_2$ receptors was 3.6 nM (log K_i = -8.4 ± 0.09, n = 6), and that of CCK-8(SO$_3$)-SAP was 3.2 nM (log K_i = -8.5 ± 0.02, n = 2). Thus, the conjugation of saporin to CCK-8(SO$_3$) does not significantly alter the affinity of CCK-8(SO$_3$) for CCK$_2$ receptors and should be effective in the targeted-lesion of CCK$_2$-expressing cells by CCK mediated internalization of saporin. Furthermore, based on the saturation analysis of [^{125}I]CCK-8(SO$_3$) for the hCCK$_1$ and hCCK$_2$ receptors, the peptide has similar affinity for the two receptor types (Kd values are 1.9 ± 0.2 nM and 1.3 ± 0.4 nM for hCCK$_1$ and hCCK$_2$ receptors, respectively). It is reasonable to predict that CCK-8(SO$_3$)-SAP has similar affinity for both CCK receptor types, while the non-sulfated CCK-8-SAP is likely to be selective for the CCK$_2$ receptors, albeit with lower affinity (56 nM; see *Targeting Trends* 4(3):5). It should be noted also that the sulfated group on CCK-8(SO$_3$) may be hydrolyzed upon storage; thus the affinity of the toxin conjugate should be verified experimentally prior to application.

We evaluated the effect of CCK-8(SO$_3$)-SAP on CCK$_2$ receptor-expressing cells in the rostral ventromedial medulla (RVM) of rats by the stereotaxic microinjection of a single dose of CCK-8(SO$_3$)-SAP (3 pmoles, bilaterally at 1.5 pmoles in 0.5 μL per side) into the RVM of anesthetized rats. Separate groups of rats were given the same dose of either saporin or CCK-8(SO$_3$) as control. All animal use and procedures were reviewed and approved by IACUC. Twenty-eight days after the RVM

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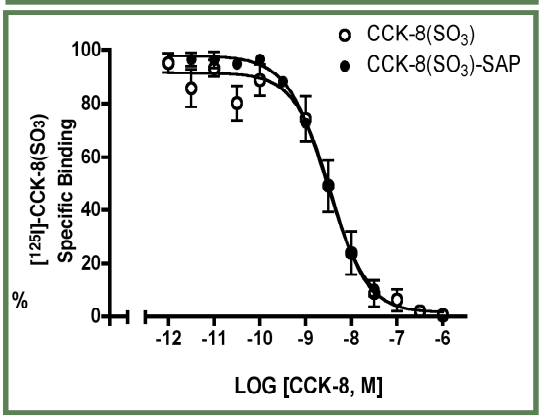
Figure 1.
[^{125}I]-CCK-8(SO$_3$) / Ligand competition in transfected HEK 293 cells that express hCCK$_2$ receptors.
Data represent mean % standard error from at least 2 independent experiments. Non-specific binding was defined by the amount of [^{125}I]-CCK-8(SO$_3$) bound in the presence of 1 μM CCK-8(SO$_3$).



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Denise Higgins, Editor

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