Targeting Tests: CCK-SAP in Binding Studies

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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 receptors types, CCK_1 and CCK_2, based on radioligand binding analysis using [^{125}I]CCK8(SO_3) in transfected HEK293 cells that express either the human CCK_1 or human CCK_2 receptors (Table 1). On the other hand, the non-sulfated form of CCK-8 exhibits lower affinity for both CCK receptor types when compared with that of the sulfated form. The non-sulfated form is also moderately selective for the CCK_2 receptors (Table 1).

Table 1

Ligand	Affinity (K_i, nM)	
	$hCCK_1$	$hCCK_2$
CCK8(SO_3) (sulfated)	0.8	1.5
CCK8 (non-sulfated)	800	125
CCK8-saporin (non-sulfated)	ND*	56
* ND; not determined. All data from at least 3 independent determinations.		

Table 1. Affinity of CCK-8 or CCK-8-saporin for the human CCK_1 and CCK_2 receptors based on competitive inhibition of [^{125}I]CCK-8 binding to HEK293 cells that stably express $hCCK_1$ or $hCCK_2$ receptors.

A covalent conjugate of non-sulfated CCK-8 to saporin (CCK-SAP) was synthesized and evaluated for the toxin conjugate's affinity for the human CCK receptors in transfected HEK293 cells (Figure 1 and Table 1). CCK-SAP exhibits similar affinity for the $hCCK_2$ receptors as non-sulfated CCK-8. The affinity of CCK-SAP for the $hCCK_1$ receptors was not determined because, based on the findings seen in the CCK_2 receptors, it is likely that the affinity of CCK-SAP for the CCK_1 receptors would be similar to that of non-sulfated CCK-8, and the cost of such assays would be prohibitive due to the high concentrations of CCK-SAP needed. Conjugation of saporin to CCK-8 does not significantly alter the affinity of CCK-8 for CCK receptors and should therefore be effective in targeted-lesion of CCK_2 expressing cells by CCK-mediated internalization of saporin.

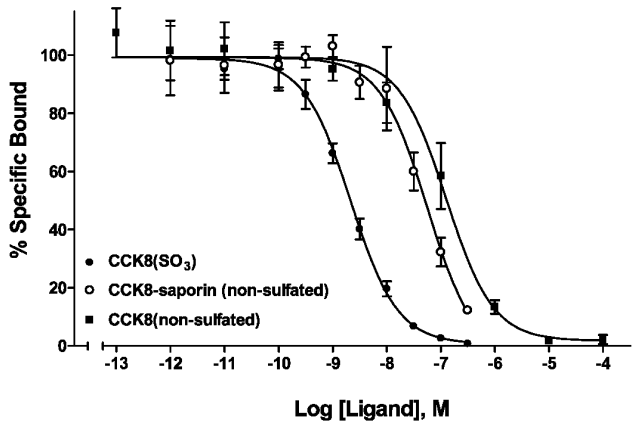

Figure 1. [^{125}I]-CCK-8(SO_3) / Ligand competition in transfected HEK 293 cells that express $hCCK_2$ receptors. Data represent mean ± S.E.M. of three independent experiments. Non-specific binding was defined by the amount of [^{125}I]-CCK-8(SO_3)(specific activity: 1200 μCi/mmol) bound in the presence of 1 μM CCK-8(SO_3).

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