A Conantokin Peptide Con-T[M8Q] Inhibits Morphine Dependence with High Potency and Low Side Effects

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Opioid receptor binding assay

Ligand binding experiments were carried out with [^{3}H]diprenorphine for opioid receptors as described previously (1,2). Competition inhibition by peptides and morphine of [^{3}H]diprenorphine binding to opioid receptors was performed in the absence or presence of various concentrations of each drug. Binding was carried out in 50mM Tris.HCl buffer (pH7.4) at 37℃ for 30min in triple in a final volume of 0.5ml with 30 μg of membrane protein prepared from CHO cell expressing human κ-, rat μ- or rat δ- opioid receptors. Naloxone (10μM) was used to define nonspecific

binding. Bound and free [^{3}H]diprenorphine were separated by filtration under reduced pressure with GF/B filters. Radioactivity on filters was determined by liquid scintillation counting.

Table S1. The binding ability of con-T[M8Q] to opioid receptor

Opioid receptor	μ-receptor	K-receptor	δ-receptor
Con-T[M8Q] (μM)	5	5	5
Inhibition (%)	13.1 ±0.1	7.1 ±0.8	0

Table S2. The primers and sequences used for qRT-PCR

Gene	Primer sequences	Annealing temperature℃
GAPDH	F: CAA GGC TGA GAA TGG GAA G	58
	R: TGG TGA AGA CGC CAG TAG A	58
GluN2B	F: ACT AAC TAT CAA TGA AGA ACG GT	60
	R: AGG AGA GGA AGA GCT ACA A	58
CAMKII-α	F: ATC GCC TAT ATC CGC ATC AC	60
	R: GGA CAA AGA GCG GAT CTC TG	60
CAMKII-β	F: CAT TGT ACG CCT CCA TGA CA	58
	R: GGA TGC AGT GAC TGG CAT CA	58
CaMK-IV	F: CGG CTG ACT ACA TTT CAA GCC	56
	R: CTT CAC CGC TGC CTT AAG CTT	58
nNOS	F: AGG AGA GGA AGA GCT ACA A	56
	R: AAG ACT GAG AAC CTC ACA TT	56
PKC-γ	F: CAA CTT CAT TCC ACC TTT CAG A	50
	R: GCA TCC AGC ATC ACA TTA TCC	50

References

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