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Differential down-regulation of the human δ-opioid receptor by SNC80 and [D-Pen^2,D-Pen^5]enkephalin

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Abstract

We examined the contribution of the human δ-opioid receptor carboxyl terminal tail to (+)-4-[(α*R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC80)- and cyclic[D-Pen^2,D-Pen^5]enkephalin (DPDPE)-mediated receptor down-regulation. Both SNC80 and DPDPE mediated down-regulation of an epitope tagged human δ-opioid receptor. Truncation of the human δ-opioid receptor after Gly^{338} blocked DPDPE-mediated down-regulation. However, SNC80 mediated significant down-regulation of the truncated receptor. These findings suggest that SNC80-mediated down-regulation involves receptor domains in addition to the carboxyl terminal tail.

Keywords: δ-Opioid receptor; Down-regulation; Carboxyl terminal tail

It has been established that chronic agonist treatment causes decreased opioid receptor activation (Zadina et al., 1995). Reduced receptor expression on the cell surface, termed down-regulation, is thought to contribute to this loss of function. Chronic treatment with (+)-4-[(α*R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC80) causes down-regulation and desensitization of the human δ-opioid receptor expressed in Chinese hamster ovary (CHO) cells (Malatynska et al., 1996). Cvejic et al. (1996) demonstrated that the carboxyl terminal tail is necessary for [D-Ala^2,D-Leu^5]enkephalin (DADLE)-mediated down-regulation and the Thr^{353} located within the carboxyl terminal tail mediates down-regulation of the mouse δ-opioid receptor. However, the role of the carboxyl terminal tail of the human δ-opioid receptor on agonist-mediated down-regulation is not known since Thr^{353} is already an Ala in the human δ-opioid receptor sequence (Knapp et al., 1994). Furthermore, it is uncertain whether the mechanism mediating down-regulation of δ-opioid receptor is identical for all agonists.

We prepared myc-his epitope tagged variants of the human δ-opioid receptor. These variants represent full-length receptor (WT-myc-his) and a 34-amino acid truncation (Δ^{34}-myc-his) labeled at the carboxyl end. δ-Opioid receptor cDNA was ligated into pcDNA3.1/myc-his to create receptor constructions and transfected into CHO cells. We examined the role of the carboxyl terminal tail on SNC80- and cyclic [D-Pen^2,D-Pen^5]enkephalin (DPDPE)-mediated receptor down-regulation using these cell lines. The cells were incubated with Iscove's modified Dulbecco's medium (IMDM) ± SNC80 or DPDPE (both 500 nM) for 24 h. After incubation, cell membranes were prepared and the binding assays for δ-opioid receptors were performed using [^{3}H]naltrindole as described in the figure legend (Knapp et al., 1994). Each experiment was

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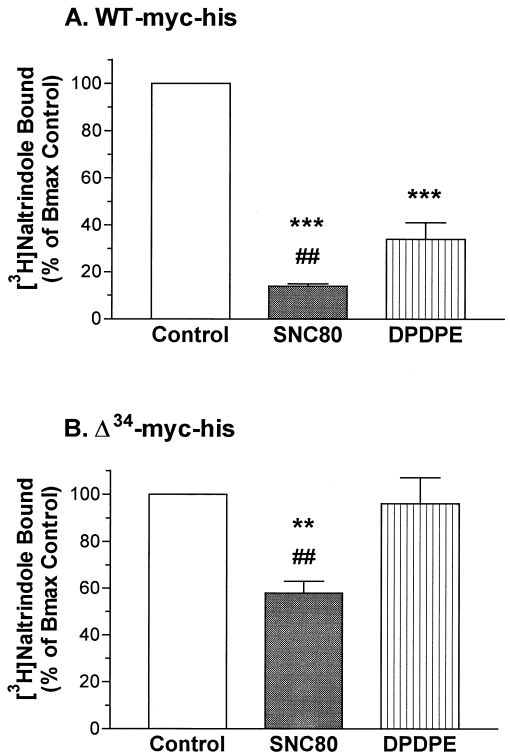

Fig. 1. Effect of chronic SNC80 or DPDPE pretreatment on the B_{max} values of specifically bound [^{3}H]naltrindole in CHO cell membranes expressing the WT-myc-his (A) and the Δ^{34}-myc-his (B). Cells were preincubated with SNC80 or DPDPE (both 500 nM) for 24 h and then rinsed with IMDM (37°C) three times. Saturation binding studies were performed using varying concentration of [^{3}H]naltrindole (0.03–0.5 nM). Briefly, membranes prepared from these cells were incubated with [^{3}H]naltrindole in Tris (50 mM)/$MgCl_2$ (5 mM) buffer, pH = 7.4, for 90 min at 30°C. Nonspecific binding was determined in the presence of 10 μM naltrexone. The B_{max} and K_d values for specifically bound [^{3}H]naltrindole were estimated by nonlinear regression analysis using the Prism ver.2 (GraphPad, San Diego, CA). Each column represents the average B_{max} value expressed as percentage of control (cells treated with IMDM only) plus the standard error of four determinations. $^{**}P < 0.01$ or $^{***}P < 0.001$ for SNC80 or DPDPE pretreatment compared with control. $^{\#\#}P < 0.01$ when SNC80 pretreatment was compared with DPDPE pretreatment.

performed in parallel with a control (cell treated with IMDM only). The apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for specifically bound [^{3}H]naltrindole were estimated by nonlinear regression analysis using the Prism ver.2 (GraphPad, San Diego, CA) and statistical significance was determined by a one-way analysis of variance (ANOVA) and a Newman–Keuls test. Statistical significance was accepted at $P < 0.05$.

Specifically bound [^{3}H]naltrindole in membranes prepared from both cell lines expressing the WT-myc-his and the Δ^{34}-myc-his appeared to be saturable and the Rosenthal analysis revealed a linear plot (data not shown), suggesting a single population of binding sites. Treatment of the WT-myc-his cells with SNC80 or DPDPE significantly decreased the B_{max} values of [^{3}H]naltrindole bound by 86% and 66%, respectively (Fig. 1A) (B_{max}: control 1410 ± 190, SNC80 pretreatment 196 ± 31, DPDPE pretreatment 479 ± 88 fmol/mg protein). In the Δ^{34}-myc-his cells, SNC80 caused a significant reduction of the B_{max} value by 42%, but there was no significant change in the B_{max} value of [^{3}H]naltrindole bound after DPDPE pretreatment (Fig. 1B) (B_{max}: control 153 ± 15, SNC80 pretreatment 90 ± 14, DPDPE pretreatment 148 ± 25 fmol/mg protein). The reduction by SNC80 was significantly different from that by DPDPE in both cell lines. The loss of specifically bound [^{3}H]naltrindole was not due to insufficient wash out of the unlabeled agonist since the K_d values for [^{3}H]naltrindole were not significantly different after treatment with SNC80 and DPDPE in both the WT-myc-his (K_d: control 117 ± 26, SNC80 pretreatment 126 ± 25, DPDPE pretreatment 109 ± 31 pM) and the Δ^{34}-myc-his cells (K_d: control 86 ± 17, SNC80 pretreatment 126 ± 26, DPDPE pretreatment 84 ± 20 pM).

These data demonstrate the differential down-regulation of the human δ-opioid receptor by SNC80 or DPDPE. Truncation of the carboxyl terminal tail from the δ-opioid receptor is sufficient to attenuate receptor down-regulation induced by chronic DPDPE exposure. In contrast, significant down-regulation of the truncated human δ-opioid receptor after SNC80 pretreatment still remains. These findings indicate that regulation of the human δ-opioid receptor by chronic SNC80 exposure involves receptor domains in addition to the carboxyl terminal tail. DPDPE-mediated down-regulation is solely dependent on the carboxyl terminal tail. The differential down-regulation mediated by SNC80 and DPDPE may be related to their recognition sites for the human δ-opioid receptor since SNC80 has a unique interaction with Trp^{284} that is not shared by other δ-opioid ligands such as DPDPE (Li et al., 1995). Thus, the molecular mechanisms responsible for SNC80- and DPDPE-mediated down-regulation of the human δ-opioid receptor are not identical.

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