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Short communication

Mutation S363A in the human δ-opioid receptor selectively reduces down-regulation by a peptide agonist

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Abstract

Chemically distinct opioid agonists have different abilities to down-regulate opioid receptors. The present study investigated the role of Ser^{363} in human δ-opioid receptor down-regulation by a δ-selective peptide- and non-peptide agonist. Cyclic[D-Pen^2,D-Pen^5]enkephalin (DPDPE)-mediated down-regulation was significantly attenuated by a S363A mutation. In contrast, this mutation had no effect on down-regulation by (+)-4-[(α*R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]*N*,*N*-diethylbenzamide (SNC80). These results demonstrate that the molecular mechanism of the human δ-opioid receptor down-regulation is agonist-specific.

Keywords: δ-Opioid receptor; Trafficking, agonist-directed; Down-regulation

Human δ-opioid receptor down-regulation is thought to be involved in the development of analgesic drug tolerance (Fleming and Taylor, 1995). Agonist-activation of G-protein-coupled receptors is typically followed by receptor phosphorylation and recruitment of β-arrestins, leading to the termination of G-protein signaling. In addition, β-arrestin binding facilitates receptor endocytosis via a clathrin- and dynamin-dependent mechanism. The internalized receptors are subsequently trafficked into lysosomes for degradation (down-regulation), or to recycling vesicles for re-incorporation into the plasma membrane. Consequently, the intracellular trafficking of the receptor participates in the regulation of receptor signaling.

It has been demonstrated that the fate of the internalized G-protein-coupled receptors is both receptor- and cell type-specific (Tsao and von Zastrow, 2000). We have previously found that the trafficking of the human δ-opioid receptor is also agonist-specific. In Chinese hamster ovary (CHO) cells stably expressing a human δ-opioid receptor, the non-peptide agonist (+)-4-[(α*R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]*N*,*N*-diethylbenzamide (SNC80) down-regulates the receptor more efficiently than the peptide agonist cyclic[D-Pen^2,D-Pen^5]enkephalin (DPDPE) (Okura et al., 2003). Furthermore, truncation of the carboxyl-terminus at Gly^{338} completely abolishes DPDPE-, but not SNC80-mediated down-regulation (Okura et al., 2000). These results led to the hypothesis that structurally distinct agonists utilize specific receptor domains to regulate receptor trafficking; namely, DPDPE, but not SNC80, requires an intact carboxyl-terminus to down-regulate the human δ-opioid receptor. In the present study, we investigated the role of Ser^{363}, the primary phosphorylation site in the distal C-terminal domain of the δ-opioid receptor (Kouhen et al., 2000), in agonist-directed trafficking.

The human δ-opioid receptor was subcloned in pcDNA3 expression vector. A mutation (Ser^{363} to Ala) was introduced using the Quick Change site-directed mutagenesis method (Stratagene). The presence of the Ser^{363} to Ala mutation was verified by nucleotide sequencing at the University of Arizona sequencing facility. A stable cell line expressing the human δ-opioid receptor (S363A) mutant

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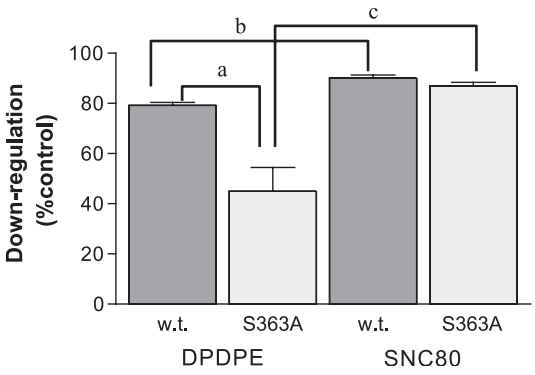

Fig. 1. Mutation of Ser^{363} to Ala in the human δ-opioid receptor specifically attenuates down-regulation by DPDPE. CHO cells stably expressing the wild-type (w.t.) or (S363A) mutant human δ-opioid receptors were treated for 24 h with 500 nM DPDPE or 500 nM SNC80. After three washes, cell membranes were prepared and [^{3}H]naltrindole (0.5 nM) binding was measured. Down-regulation was defined as a decrease in specific binding per microgram membrane protein after the agonist treatment and expressed as a percentage of the buffer-treated control. Data shown represent means±S.E.M. of at least four independent experiments. (a) Down-regulation by DPDPE in the (S363A) mutant is significantly attenuated (45±9.5%) as compared to the wild-type human δ-opioid receptor (79±1.2), $^{**}P<0.01$. (b) The wild-type human δ-opioid receptor is more effectively down-regulated by SNC80 (90±1.3) then by DPDPE (79±1.2), $^{***}P<0.001$. (c) The human δ-opioid receptor (S363A) mutant is more effectively down-regulated by SNC80 (87±1.4) then by DPDPE (45±9.5%), $^{**}P<0.01$.

was generated by transfecting the pcDNA3 construct into CHO cells via FuGENE (Roche) and G418 selection. The recombinant CHO cell line expressing the human δ-opioid receptor has been previously characterized (Malatynska et al., 1996). The recombinant CHO cell lines expressing the wild-type human δ-opioid receptor, or the human δ-opioid receptor (S363A) mutant (B_{max}=2640 fmol/mg, K_d=110 pM) were treated with 500 nM DPDPE or 500 nM SNC80 for 24 h, and cell membranes were prepared as described (Okura et al., 2003). To determine the amount of remaining receptors, specific [^{3}H]naltrindole binding to cell membranes was measured. Down-regulation was defined as a decrease in specific binding per microgram membrane protein after agonist treatment. Data were analyzed using GraphPad Prizm 3.02 software.

The results indicate that the S363A mutation has a differential effect on human δ-opioid receptor down-regulation mediated by two structurally different full agonists, DPDPE and SNC80 (Fig. 1). After 24 h DPDPE treatment, down-regulation of the human δ-opioid receptor (S363A) mutant was significantly attenuated (45±9.5%) compared to that of the wild-type human δ-opioid receptor (79±1.2, $P<0.01$). In contrast, SNC80-mediated down-regulation was virtually unaffected by the mutation (87±1.4% for the (S363A) mutant and 90±1.3% for the wild-type human δ-opioid receptor). In addition, in both the wild-type and the (S363A) mutant human δ-opioid receptor, SNC80 was significantly more effective in down-regulating the receptor than DPDPE.

The current data confirm our previous finding that human δ-opioid receptor down-regulation is agonist-specific. Moreover, in the present study, we identify Ser^{363} as a C-terminal residue critical for DPDPE-mediated down-regulation. In contrast, this residue has no effect on down-regulation by SNC80. Phosphorylation of the COOH-terminus is important for receptor internalization (Whistler et al., 2001). In the mouse δ-opioid receptor, receptor phosphorylation is hierarchical with Ser^{363} being the primary phosphorylation site upon DPDPE treatment (Kouhen et al., 2000). The carboxyl-terminal residues are also involved in post-endocytic sorting of the δ-opioid receptor to degradation pathways upon chronic peptide agonist treatment (Trapaidze et al., 2000). Significantly, in the present study, we demonstrate that, although Ser^{363} has an important role in the human δ-opioid receptor down-regulation by DPDPE, it is not essential for receptor down-regulation by SNC 80. Using a truncated human δ-opioid receptor, we previously demonstrated that, in addition to the carboxyl-tail, other domains are involved in SNC80-mediated down-regulation (Okura et al., 2003). This additional mechanism is able to compensate for the lack of Ser^{363} phosphorylation in the (S363A) mutant receptor.

In summary, our results show that mutation of Ser^{363} to Ala in the human δ-opioid receptor selectively attenuates receptor down-regulation by DPDPE. Elucidation of agonist-specific mechanisms of human δ-opioid receptor down-regulation may provide a tool to identify the sorting signals involved in receptor trafficking. Since receptor down-regulation is implicated in the development of drug tolerance, novel analgesics with selective trafficking properties may be developed that produce less tolerance.

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References

Fleming, W.W., Taylor, D.A., 1995. Cellular mechanisms of opioid tolerance. In: Tseng, L. (Ed.), The Pharmacology of Opioid Peptides. Harwood Academic Publishers, Singapore.

Kouhen, O.M., Wang, G., Solberg, J., Erickson, L.J., Law, P.Y., Loh, H.H., 2000. Hierarchical phosphorylation of δ-opioid receptor regulates agonist-induced receptor desensitization and internalization. J. Biol. Chem. 275, 36659–36664.

Malatynska, E., Wang, Y., Knapp, R.J., Waite, S., Calderon, S., Rice, K., Hruby, V.J., Yamamura, H.I., Roeske, W.R., 1996. Human delta opioid receptor: functional studies on stably transfected Chinese hamster ovary cells after acute and chronic treatment with the selective nonpeptidic agonist SNC-80. J. Pharmacol. Exp. Ther. 278, 1083–1089.

Okura, T., Cowell, S.M., Varga, E.V., Burkey, T.H., Roeske, W.R., Hruby,

V.J., Yamamura, H.I., 2000. Differential down-regulation of the human delta-opioid receptor by SNC80 and [D-Pen(2),D-Pen(5)enkephalin]. Eur. J. Pharmacol. 387, R11–R13.

Okura, T., Varga, E.V., Hosohata, Y., Navratilova, E., Cowell, S.M., Rice, K., Nagase, H., Hruby, V.J., Roeske, W.R., Yamamura, H.I., 2003. Agonist-specific down-regulation of the human δ-opioid receptor. Eur. J. Pharmacol. 459, 9–16.

Trapaidze, N., Cvejic, S., Nivarthi, R.N., Abood, M., Devi, L.A., 2000. Role for C-tail residues in delta opioid receptor downregulation. DNA Cell Biol. 19, 93–101.

Tsao, P.I., von Zastrow, M., 2000. Type-specific sorting of G protein-coupled receptors after endocytosis. J. Biol. Chem. 275, 11130–11140.

Whistler, J.L., Tsao, P., von Zastrow, M., 2001. A phosphorylation-regulated brake mechanism controls the initial endocytosis of opioid receptors but is not required for post-endocytic sorting to lysosomes. J. Biol. Chem. 276, 34331–34338.