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European Journal of Pharmacology 371 (1999) 235–240

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Differential desensitization of human δ-opioid receptors by peptide and alkaloid agonists

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Received 3 December 1998; received in revised form 9 March 1999; accepted 12 March 1999

Abstract

The efficacy of different opioid agonists to induce acute desensitization of the human δ-opioid receptor-mediated inhibition of cAMP accumulation was investigated in the neuroblastoma cell line SK-N-BE, which endogenously expresses these receptors. While etorphine, a non-selective alkaloid agonist, caused 50% desensitization after a 30-min incubation, the same treatment in the presence of the selective peptide agonists, DPDPE ([D-Pen^2,D-Pen^5]enkephalin) and deltorphin I (Tyr-D-Ala-Phe-Asp-Val-Val-Gly), almost totally desensitized the δ-opioid receptor-mediated inhibition of adenylyl cyclase. When SK-N-BE cells were prechallenged either with alkaloid or peptide agonist, we observed a cross-desensitization that was less marked when cells were pretreated with peptide agonists and then challenged with etorphine. Taken together, these results demonstrate that human δ-opioid receptors are differentially desensitized by alkaloid and peptide agonists.

Keywords: δ-Opioid receptor; Alkaloid agonist; Peptide agonist; Desensitization; Adenylyl cyclase inhibition

1. Introduction

Opioid agonists, such as morphine or its derivatives, are currently used in the management of chronic pain (Herz, 1993). Pharmacological and molecular data led to the identification of three major classes of opioid receptors, namely μ, δ and κ (for review, see Reisine, 1995). While the μ-opioid receptor represents the primary target of morphine, which produces antinociception (Matthes et al., 1996), δ-opioid receptors have also been demonstrated to mediate analgesia (Sanchez-Blazquez et al., 1997; Narita et al., 1997a). These receptors have been reported to be critical in the development of tolerance to morphine (Suzuki et al., 1997), which could be initiated by the desensitization of opioid receptors (Loh et al., 1988).

Phosphorylation of opioid receptors by second messenger-dependent kinases (Narita et al., 1997b) or by G protein-coupled receptor kinases (GRK) (Pei et al., 1995; Hasbi et al., 1998) was demonstrated to be the major step triggering desensitization by uncoupling the receptors from their associated G proteins. Recent reports have pointed out that opioid agonists have distinct potencies to desensitize and internalize μ- (Blake et al., 1997a), δ- (Bot et al., 1997) as well as κ-opioid receptors (Blake et al., 1997b) when transfected in host cells.

In the current study, we investigated the regulation of adenylyl cyclase by human δ-opioid receptors, endogenously expressed in the neuroblastoma cell line SK-N-BE (Polastron et al., 1994), in the presence of a non-selective alkaloid agonist, etorphine, and in the presence of selective peptide agonists, DPDPE and deltorphin I. Our major findings indicate that the δ-opioid receptors are more rapidly desensitized by peptide agonists than by etorphine.

2. Material and methods

2.1. Cell culture

SK-N-BE cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum, 2 mM L-glutamine and 1% antibiotic–antimycotic solution.

Abbreviations: DPDPE, [D-Pen^{2,5}]enkephalin and Deltorphin I, [Tyr-D-Ala-Phe-Asp-Val-Val-Gly]

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00180-6

2.2. cAMP accumulation measurement

SK-N-BE cells were seeded in 24-well plates at a density of 1.5 10^5 cells per well in a culture medium supplemented with 0.6 μCi [^{3}H]adenine and incubated overnight. cAMP accumulation was determined in the presence of 0.5 mM isobutylmethylxanthine (IBMX) and 40 μM forskolin and in the absence or in the presence of agonists for 5 min at 37°C. The reaction was stopped by addition of 250 μl trichloroacetic acid 5% and the separation of [^{3}H]cAMP was realized by chromatography on acid alumina columns as described previously (Allouche et al., 1996).

The basal level of cAMP was determined in the absence of forskolin or any drug and subtracted from stimulation (forskolin) and inhibition (forskolin + agonist) levels. Data represent the inhibition of forskolin-stimulated cAMP accumulation and the experiments were performed in triplicate, carried out at least three to four times with identical results.

ED_{50} values and maximal inhibitory levels of opioid agonists were determined by curve fitting of dose–response curves using SigmaPlot (Jandel Scientific) and statistical analysis was realized using Statview software.

3. Results

3.1. Inhibition of cAMP accumulation by alkaloid and peptide agonists

The ability of an alkaloid agonist, etorphine, and of peptide agonists, DPDPE and deltorphin I, over a large range of concentrations (1 nM to 10 μM), to inhibit the forskolin-stimulated adenylyl cyclase was tested in SK-N-BE cells. As shown in Fig. 1, etorphine, DPDPE and deltorphin I were all effective in inhibiting cAMP accumulation in a concentration-dependent manner with an ED_{50} of 0.6 ± 0.17, 12.5 ± 5 and 0.35 ± 0.06 nM, respectively. DPDPE and deltorphin I produced maximal inhibition (40%) at a concentration of 100 and 10 nM, respectively, while the maximal inhibition of cAMP accumulation induced by 100 nM etorphine was significantly higher than that produced by peptide agonists (50.2 ± 2.6% vs. 41.7 ± 3.3% for DPDPE and 38.4 ± 2.4% for deltorphin I, analysis of variance (ANOVA), Scheffe test, $P < 0.05$). The ability of all agonists to inhibit adenylyl cyclase activity was blocked by 10 μM of naloxone for 100 nM DPDPE and etorphine and 500 μM naloxone for 10 nM deltorphin I (Fig. 1).

3.2. Differential desensitization of δ-opioid receptor-mediated inhibition of adenylyl cyclase by alkaloid and peptide agonists

We examined the efficacy of etorphine, DPDPE and deltorphin I to acutely desensitize the δ-opioid receptor-mediated inhibition of adenylyl cyclase. SK-N-BE cells were exposed for various times (0, 5, 10, 15 and 30 min) in the presence of a maximally effective concentration of each agonist. As soon as 5 min, we could observe a significant reduction in the inhibitory action of all agonists (Fig. 2). Interestingly, while pretreatment with peptide agonists for 30 min produced a robust desensitization of

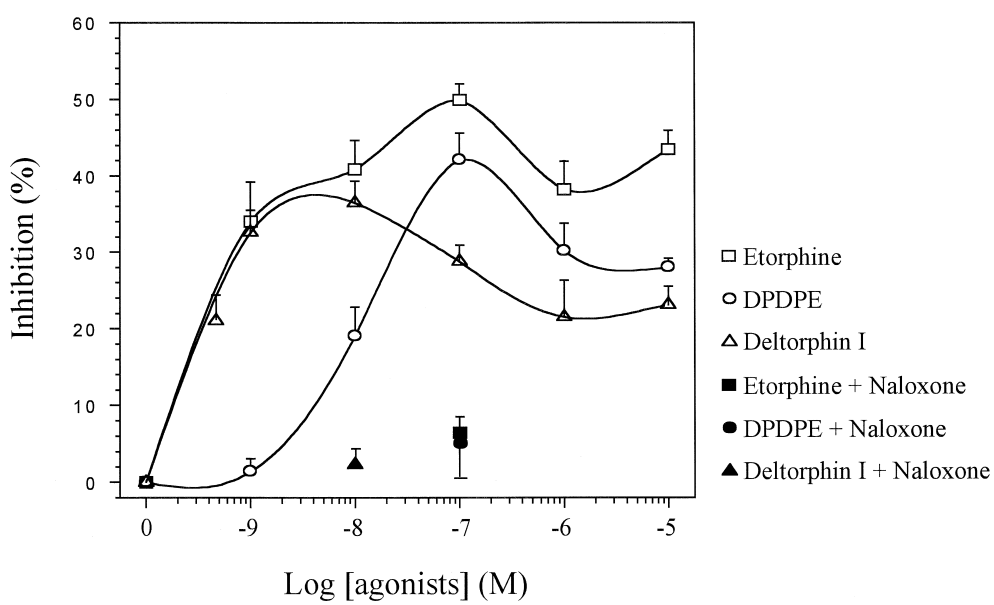

Fig. 1. Concentration-dependent inhibition of cAMP accumulation by alkaloid and peptide agonists in SK-N-BE cells. Cell monolayers were incubated in the presence of 0.5 mM IBMX, 40 μM forskolin and different concentrations of deltorphin I, etorphine and DPDPE for 5 min at 37°C. The specificity of the agonist-induced inhibition of adenylyl cyclase was tested by the addition of 10 μM of naloxone for 100 nM of DPDPE and etorphine and 0.5 mM naloxone for 10 nM deltorphin I. The reaction was stopped by addition of 5% trichloroacetic acid and the cAMP was measured as described in Section 2. Data are mean ± S.D. (n = 3–4).

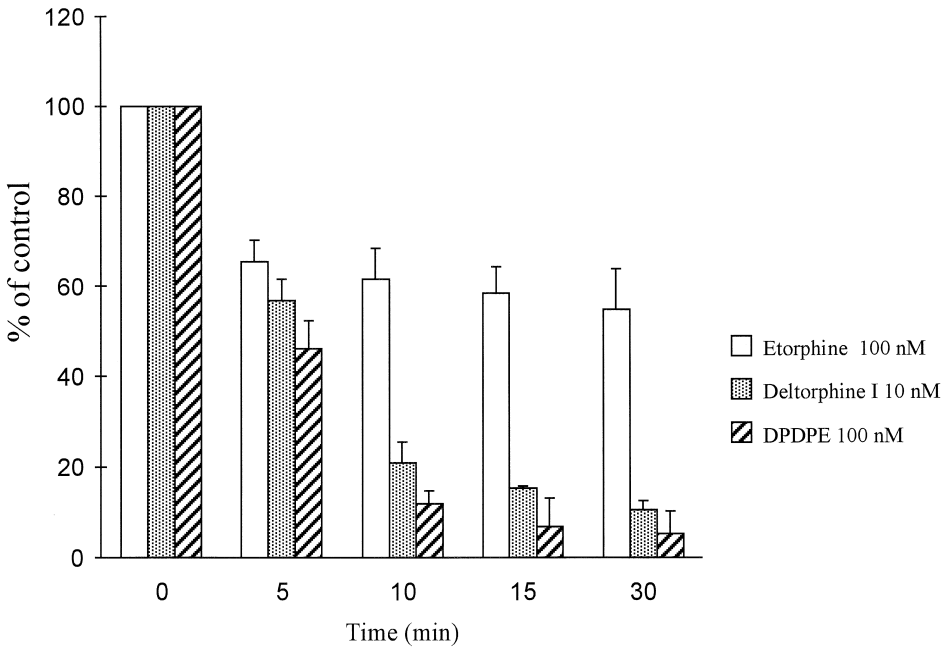

Fig. 2. Differential efficacy of alkaloid and peptide agonists to induce desensitization of δ-opioid receptor-mediated adenylyl cyclase inhibition. SK-N-BE cells were pretreated either with 100 nM etorphine, 100 nM DPDPE or 10 nM deltorphin I for various times. At the end of the pretreatment, we determined the ability of each agonist to inhibit cAMP accumulation. Opioid agonist-induced inhibition of adenylyl cyclase was taken as 100% in naive cells and results are presented as percentages of those of naive cells. Data are means ± S.D. of three different experiments performed in triplicate.

90%, prechallenging SK-N-BE cells with 100 nM etorphine during the same period induced a more limited desensitization of 55 ± 9% (Fig. 2). The reduction of the inhibition of cAMP accumulation clearly reflected the desensitization of δ-opioid receptors since the addition of fresh agonist after a 30-min exposure could not restore the inhibition of cAMP accumulation (data not shown).

3.3. Cross-desensitization between alkaloid and peptide agonists

The cross-desensitization between peptide and alkaloid agonists was further examined. As the withdrawal of agonist after a pretreatment induced an 'overshoot' of forskolin-stimulated adenylyl cyclase (Table 1), the second agonist was added without a wash-out phase and cAMP accumulation was then measured. Pretreatment of SK-N-BE cells with 100 nM etorphine for 30 min, which caused a desensitization of 50% as compared to control, totally blocked the peptide agonist-induced inhibition of cAMP accumulation (Fig. 3A and B), as shown by the absence of additional inhibition when DPDPE or deltorphin I was added to the medium. Reciprocally, when the neuroblastoma cells were pretreated with either 100 nM DPDPE or 10 nM deltorphin I for 30 min, a treatment that decreased their inhibitory actions, the responsiveness to etorphine was significantly reduced as compared to that of naive cells challenged with etorphine (Fig. 3C and D) but etorphine could still inhibit cAMP accumulation.

Table 1
Compensatory changes of forskolin-stimulated adenylyl cyclase activity following opioid agonist treatment

	Control FSK (%)	Agonist pretreatment FSK (%)
Etorphine	100	115 ± 1.9
DPDPE	100	128 ± 11.6
Deltorphin I	100	131 ± 7

SK-N-BE cells were pretreated in the presence of either DMEM/HEPES (control), 100 nM etorphine, 100 nM DPDPE or 10 nM deltorphin I for 30 min.
Cells were rapidly washed with DMEM/HEPES and the cAMP accumulation was determined in the presence of 40 μM forskolin.
The values of forskolin-stimulated adenylyl cyclase in control cells were defined as 100%.
Data represent means ± S.D. of three different experiments.

4. Discussion

In the current study, we investigated the functional consequences of exposure to an alkaloid, etorphine, and the peptide agonists, DPDPE and deltorphin I, on the regulation of adenylyl cyclase activity by human δ-opioid receptors in SK-N-BE cells. This cell line was demonstrated to express only δ-opioid receptors (Polastron et al., 1994) and, more precisely, the pharmacological subtypes δ1 and δ2. Using pharmacological (i.e., 'selective' ligands) and molecular tools, we found evidence that the two

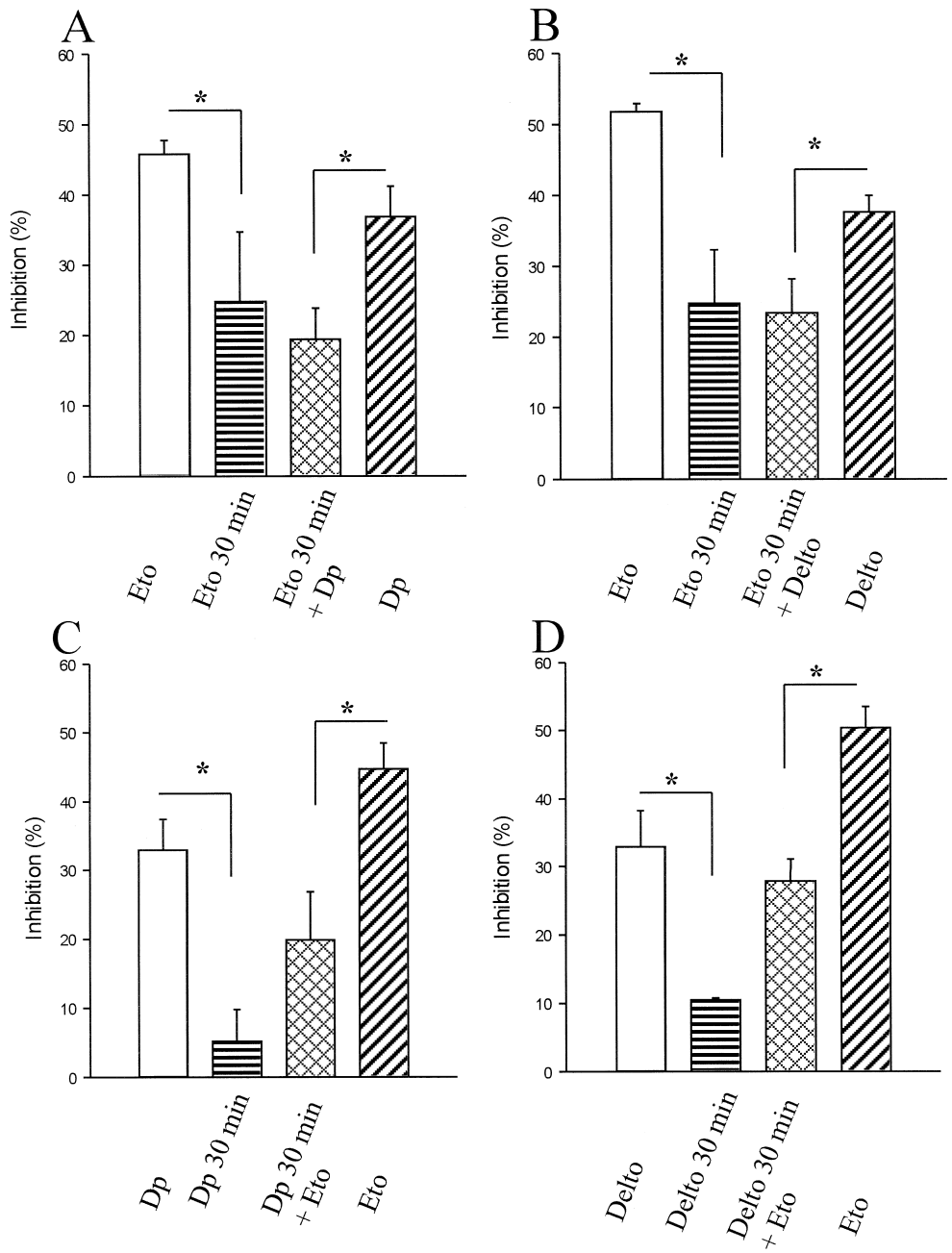

Fig. 3. Cross-desensitization between alkaloid and peptide agonists. Cells were pretreated with 100 nM etorphine (Eto) for 30 min and then challenged with either 100 nM DPDPE (Dp) (panel A) or 10 nM deltorphin I (Delto) (panel B) for intracellular cAMP determination. SK-N-BE cells were pretreated with either 100 nM DPDPE (panel C) or 10 nM deltorphin I (panel D) for 30 min and the etorphine-induced inhibition of adenylyl cyclase was determined. Data represent means ± S.D. of three different experiments performed in triplicate. Statistical significance was determined by ANOVA followed by Scheffe test. *, $P < 0.05$.

subtypes could represent a unique receptor with different binding sites for the 'selective' δ1-opioid and δ2-opioid receptor agonists DPDPE and deltorphin I, respectively (S. Allouche et al., submitted). Indeed, we observed cross-desensitization between DPDPE and deltorphin I after sustained activation of δ receptors. Moreover, when the two agonists were applied together, we could not detect any additional inhibition of adenylyl cyclase.

A significant finding of the present study was the differential desensitization of the δ-opioid receptor-mediated inhibition of adenylyl cyclase induced by alkaloid agonist, on one hand, and peptide agonists, on the other hand. These differences could be related to the partial agonist behaviour of etorphine whereas DPDPE and deltorphin I acted as full agonists. This hypothesis is not supported by our data showing that etorphine exhibited a

slightly greater efficacy in inhibiting cAMP accumulation than peptide agonists, thus, indicating that the alkaloid agonist is a full agonist.

The desensitization was rapid, and a significant reduction of the inhibition of cAMP accumulation induced by all agonists was detectable as soon as 5 min. Interestingly, when micromolar concentrations of opioid agonists were used, their inhibitory action on adenylyl cyclase was significantly reduced. This probably reflects the rapid desensitization of δ-opioid receptors saturated by agonists. In contrast to etorphine, the efficacy of peptide agonists to inhibit cAMP accumulation was almost totally abolished after a 15- to 30-min pretreatment. An identical observation was reported by Ling et al. (1998) for Chinese hamster ovary (CHO) cells stably expressing human κ-opioid receptors. These authors observed that dynorphin A-(1–13), a natural opioid peptide, induced a much stronger desensitization of the opioid receptor-mediated extracellular acidification than the alkaloid agonist etorphine.

The molecular basis underlying the ability of alkaloid and peptide agonists to differentially induce acute desensitization of opioid receptors is not clearly established. Recent studies have demonstrated that peptide and alkaloid ligands bind to δ- (Meng et al., 1996), κ- (Xue et al., 1994) and μ-opioid receptors (Fukuda et al., 1995) at different sites. Peptides were proposed to interact with the extracellular loops and transmembrane domain interface of the δ-opioid receptor while the small alkaloid ligands bound deeper, in the pocket formed by the transmembrane domains (Meng et al., 1996). Thus, a possible explanation for the differential desensitization is that activation of δ-opioid receptors by alkaloid and peptide agonists induces different conformations that vary in their susceptibility to phosphorylation by kinases. Such a difference between alkaloid and peptide agonists has been recently reported by Chakrabarti et al. (1998), who observed that the μ-opioid receptor was phosphorylated by cAMP-dependent protein kinase in the presence of morphine while DAMGO ([D-Ala^2, Mephe^4, Gly-ol^5]enkephalin) was unable to stimulate phosphorylation by this kinase.

In SK-N-BE cells, we previously demonstrated that the desensitization of δ-opioid receptors by etorphine involved their phosphorylation by a member of the G protein-coupled receptor kinase family (Hasbi et al., 1998), and probably by G protein-coupled receptor kinase 2 since it is the only member of this kinase family expressed in our cellular model (in preparation). Another explanation is that the recruitment of the G protein-coupled receptor kinase from the cytosol to the plasma membrane, which involves free Gβγ proteins (Pitcher et al., 1995), is more efficient in the presence of peptide agonists than in the presence of etorphine. In vitro studies have shown that the affinity of the G protein-coupled receptor kinase 2 for Gβγ proteins depends on βγ isoforms. When transfected in host cells, this kinase was demonstrated to specifically interact with Gβ1 and Gβ2 isoforms (Daaka et al., 1997). In [α-^{32}P]azidoanilide-GTP photolabelling studies, we observed in our cellular model that etorphine and peptide agonists stimulated different G proteins. Thus, one can assume that DPDPE and deltorphin I could release Gβγ dimers that interact rapidly with the G protein-coupled receptor kinase 2 while different Gβγ dimers with a lower affinity for this kinase would be released upon binding of etorphine to δ-opioid receptors.

Interestingly, we observed that etorphine pretreatment totally blocked the peptide agonist-induced inhibition of adenylyl cyclase and that the reverse cross-desensitization was less marked because prechallenge with DPDPE and deltorphin I reduced but did not block the cAMP inhibition produced by etorphine. These data strengthen the idea that peptide and alkaloid agonists interact differently at the human δ-opioid receptor. This observation is in good agreement with the differences between alkaloid and peptide ligands reported by Von Zastrow et al. (1993) in neuroblastoma NG 108-15 cells. These authors showed that pretreatment with etorphine induced a selective inhibition of peptide agonist binding while the ability of alkaloid ligands to interact with the mouse δ-opioid receptor was not affected.

In summary, our results show that chemically different opioid agonists produce different patterns of desensitization of human δ-opioid receptors. This different regulation would suggest that peptide agonists could contribute to the development of tolerance in humans. Knowledge of the molecular mechanisms underlying this differential desensitization by distinct agonists would provide insight into opioid tolerance.

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