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Immunomodulatory responses to δ-opioid receptor ligands in human lymphocytes

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Abstract

In phytohemagglutinin (PHA) activated human peripheral blood mononuclear cells, [^{3}H]thymidine uptake and interferon γ production were increased by the δ-opioid receptor agonist, deltorphin-I (10^{-14}–10^{-10} M) and by the δ-opioid antagonist naltrindole (10^{-13}–10^{-9} M). Combination of 10^{-9} M naltrindole with deltorphin-I (10^{-12}–10^{-8} M) significantly inhibited the proliferative response but did not affect interferon production. © 1997 Elsevier Science B.V.

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It is well established that opiate alkaloids and opioid peptides affect host defense mechanisms by modulating the activation, proliferation and effector function of mononuclear cells (Sibinga and Golstein, 1988; Rouveix, 1992). However, the mode of action of opioids on immune cells remains unclear and it has been suggested that it might differ from the opioid pharmacology classically described for neurons. Using the selective δ-opioid receptor agonist, deltorphin-I (Espamer et al., 1989), Stefano et al. (1992) detected in human granulocytes, mouse spleenocytes and invertebrate immunocytes a special δ-opioid receptor subtype that stimulated cellular adherence, conformational changes and proliferative responses. Here we studied the effects of deltorphin-I and the δ-selective opioid antagonist, naltrindole (Portoghese et al., 1988), on the proliferative response and interferon (IFN) production of resting and phytohemagglutinin (PHA)-activated human peripheral blood mononuclear cells.

Peripheral blood mononuclear cells were obtained from the heparinized venous blood of healthy humans. After centrifugation on a Ficoll-Hypaque density gradient (Boyum, 1976) and two washes with PBS, cells were suspended in RPMI 1640 medium (with 10% fetal calf serum and gentamycin 50 mg/ml) at a concentration of 1×10^6 cells/ml. Cells were cultured in the medium for 72 h at 37°C (5% CO_2) in the absence or in the presence of phytohemagglutinin (9 mg/ml) and graded opioid concentrations (0, 10^{-15} to 10^{-8}). Cultures were then incubated for 2 h with 1 μCi of [^{3}H]thymidine and then harvested onto glass fiber filters. The results are expressed as the mean cpm ± S.E.M. of triplicate cultures. Student's *t*-test was used to determine significant differences between experimental and control (without opioids) groups. Interferon titers were determined by inhibition of the Sindbis virus hemagglutinin after a single growth cycle (Stanton et al., 1981), in supernatants from parallel experiments without [^{3}H]thymidine. Interferon activity is expressed in international units.

In the absence of PHA, neither deltorphin-I nor NLT induced significant changes in [^{3}H]thymidine uptake or interferon titers. Cells cultured in the presence of deltorphin-I (10^{-14} to $10^{-8)}$ and PHA showed a significant increase in [^{3}H]thymidine uptake as compared to cells cultured with PHA alone. This increase was a bell-shaped dose-dependent phenomenon: the maximum increase (+71%) was obtained with deltorphin-I 10^{-10} M, whereas higher concentrations resulted in a small (10^{-9} M, +22%) and non-significant (10^{-8} M, +11%) increase in proliferation. The interferon content of supernatants of PHA-activated human peripheral blood mononuclear cells, cultured in the presence of deltorphin-I, was significantly higher than that of supernatants of control PHA-activated human peripheral blood mononuclear cells. At concentrations ranging between 10^{-14} to 10^{-12} M the interferon

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titers increased in parallel with [^{3}H]thymidine uptake, but at 10^{-11} and 10^{-10} M the percent increase in interferon was significantly higher (1.6 and 2.5 times) than the percent increase in [^{3}H]thymidine uptake, indicating that, at these concentrations, the increased titers of interferon measured in supernatants depended upon the increased cellular production of interferon and not on the increased number of cells producing interferon (Table 1).

To assess if these actions depend on the activation of the δ-opioid system, we performed parallel experiments in the presence of the δ-selective antagonist, naltrindole (10^{-9} M). Surprisingly, naltrindole alone significantly increased [^{3}H]thymidine uptake (+50%). Nevertheless, it abolished the increase in [^{3}H]thymidine uptake induced by a low dose deltorphin-I. Moreover, when combined with the higher concentrations of deltorphin-I (10^{-12} to 10^{-8} M), naltrindole significantly reduced [^{3}H]thymidine uptake (−28 to −36%). In contrast, naltrindole 10^{-9} M did not interfere with the deltorphin-I-induced increase in interferon titers (data not shown). Studies evaluating [^{3}H]thymidine uptake and interferon titers of PHA-activated human peripheral blood mononuclear cells cultured in the presence of increasing concentrations (10^{-15}–10^{-8} M) of naltrindole alone showed that naltrindole, at concentrations higher than 10^{-13} M, induced a non-dose-dependent increase in cell proliferation and interferon production. The present data lead us to hypothesize that naltrindole does not behave as an antagonist of deltorphin-I-induced cell proliferation, but, acting as an agonist, contributes to increase the total agonist concentration beyond those values that, based on the bell-shaped dose–response curve, are inhibitory. Moreover, the complexity of molecular mechanisms for interferon production could explain the different relation between [^{3}H]thymidine uptake and interferon production.

In conclusion, like the highly potent and selective agonist deltorphin-I, naltrindole, which acts as an antagonist at the brain δ-opioid receptor, stimulates the δ-opioid receptor in PHA-activated human peripheral blood mononuclear cells, increasing cell proliferation and interferon production. The hypothesis that yet uncloned opioid receptors might be responsible for the actions of opioid on immune cells is a reasonable possibility. Naltrindole might act as an agonist at this putative, as yet unknown receptor.

Table 1
Effects of increasing concentrations of deltorphin-I and naltrindole on PHA-activated human lymphocytes proliferative response and IFN production

Concentration (M)	[^{3}H]THD uptake		IFN titers (M)	
	cpm×10^{-3}	Δ%	U ml^{-1}	Δ%
Deltorphin-I				
0	85±7.9		430±100	
10^{-15}	84.7±10.3	−0.1	400±90	+0
10^{-14}	120.0±10 *	+41	600±100	+39
10^{-13}	125.0±14 *	+47	600±100	+39
10^{-12}	132.0±15.9 *	+55	700±100 *	+62
10^{-11}	142.5±13.5 *	+68	900±125 *	+109
10^{-10}	145.7±16.9 *	+71	1200±160 *	+179
10^{-9}	104.5±13.6	+22	500±90	+16
10^{-8}	95.0±10.6	+12	450±90	+4
Naltrindole				
0	86±10		600±130	
10^{-15}	80±13	−7	400±100	−34
10^{-14}	121±11 *	+40	500±80	−17
10^{-13}	104±12	+20	600±100	0
10^{-12}	111±9	+29	600±90	0
10^{-11}	126±13 *	+46	1500±200 *	+150
10^{-10}	121±10 *	+40	700±100	+17
10^{-9}	133±16 *	+55	1024±150	+70
10^{-8}	115±9.5	+34	500±100	−17

Values are means±S.E.M from 3 experiments. Δ%: percent variation. The control levels for interferon (IFN) production ranged from 200 to 850 U/ml.
* $P < 0.05$.

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