in the case of 4 and 5. However, the IC_{50} of 9 in L1210 cells was equicytotoxic to Clomesone.[4b]

In summary, we have identified alkylating organoselenones as potential lead compounds for development as potential antitumor agents, based on their novel selenium–carbon bond cleavage, useful reactivities, good solubilities, high nucleophilic selectivities, and highly potent antiproliferative activities against human and murine leukemic cell lines. Noteworthy is the finding of a lack of cross-resistance to L1210/L-PAM by 2-chloroethyl arylselenones. An areneseleninate, a probable metabolite from an arylselenone, did not account for the enhanced cell growth inhibition, which is probably related to cross-link formation.

Acknowledgment. We are indebted to Thomas H. Corbett at Wayne State University for the disk diffusion assay, to Daniel Von Hoff for the BACTEC screen, and to Daniel P. Griswold, Jr., at the Southern Research Institute for the L1210 cell lines.

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Received February 27, 1989

Naltrindole 5′-Isothiocyanate: A Nonequilibrium, Highly Selective δ Opioid Receptor Antagonist

Sir:

Opioid ligands are among the best-known classes of physiologically active agents that interact with multiple subpopulations of opioid receptors.[1,2] Receptors that recognize such ligands have been categorized into three major types, the best documented of which are named μ, κ, and δ. The enkephalins, endorphins, and dynorphins represent some of the classes of the endogenous peptides that interact with these multiple receptor types.

Efforts to develop opioid receptor affinity labels (nonequilibrium opioid ligands) have been pursued as biochemical and pharmacologic probes to address the problem of receptor multiplicity and receptor isolation.[3] However, until very recently there were no reports of nonequilibrium, δ-selective opioid receptor antagonists that are active in vivo. Ligands such as fentanyl isothiocyanate (FIT) and its 3-methyl analogue (SUPERFIT) are useful affinity labels, but they display no nonequilibrium antagonist activity in vivo.[4] A recently designed affinity label, [D-Ala^2,Leu^5,Cys^6]enkephalin (DALCE), has been reported to bind covalently to δ receptors by a thiol–disulfide exchange mechanism and it appears to possess a pharmacologic profile in vivo consistent with nonequilibrium δ opioid receptor antagonism.[5,6]

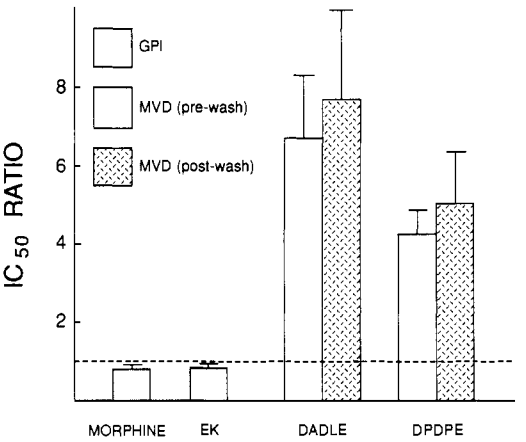
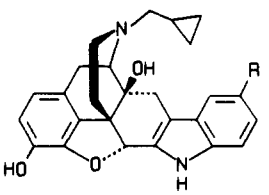


Figure 1. The antagonism of μ-, κ-, and δ-selective agonists by NTII (100 nM) in GPI and MVD preparations. Morphine and ethylketazocine (EK) were tested in the GPI, and the δ-selective ligands (DADLE and DPDPE) were evaluated in the MVD. The prewash IC_{50} values were obtained by determining the agonist IC_{50} in the presence of NTII prior to washing. Postwash IC_{50} values were obtained by thoroughly washing the preparation that was incubated with NTII and then determining the agonist IC_{50}. The IC_{50} ratio was calculated by dividing the IC_{50} of the standard agonist in the NTII-treated preparation by the IC_{50} of the agonist alone in the same tissue. The dashed line represents a ratio of 1 (no change).

Here we report on the design and synthesis of the first nonpeptide, nonequilibrium δ opioid receptor antagonist, naltrindole 5′-isothiocyanate (1, NTII), that exhibits high pharmacologic selectivity both in vitro and in vivo.



1 R = N=C=S
2 R = H
3 R = NO_2
4 R = NH_2

The design of NTII (1) was based on the attachment of the isothiocyanate group to naltrindole[7] (2, NTI), a highly selective, reversible δ opioid receptor antagonist. The isothiocyanate group is capable of reacting under physiologic conditions with NH_2, imidazole, and thiol groups.[8] Its reactivity with water or hydroxyl groups is negligible. The isothiocyanate group has been employed successfully in the design of a number of opioid receptor affinity labels.[3]

Target compound 1 was prepared by reacting naltrexone with (*p*-nitrophenyl)hydrazine to form 5′-nitroindole 3, which was reduced to 5′-amino derivative 4 and then treated with thiophosgene.[9]

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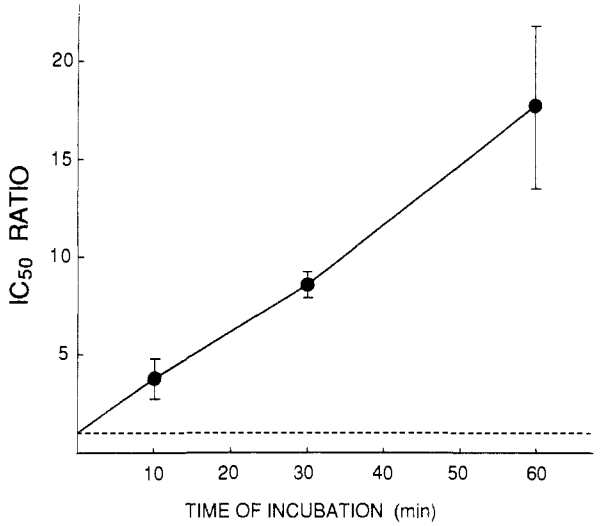

Figure 2. The irreversible antagonism of DADLE by NTII (1) (100 nmol) as a function of time in the MVD preparation. The IC_{50} ratio is the IC_{50} of DADLE in the treated MVD divided by the control IC_{50} in the same preparation. The dashed line represents a ratio of 1 (no change).

When NTII (1) (100 nM) was incubated with the mouse vas deferens preparation[10] (MVD) for 30 min and subsequently tested with the δ-selective agonists [D-Ala^2,D-Leu^5]enkephalin[11] (DADLE) and [D-Pen^2,D-Pen^5]enkephalin[12] (DPDPE), their IC_{50} values were shifted to a higher concentration as indicated by IC_{50} ratios of greater than 1 (Figure 1). The irreversible antagonism of NTII was confirmed by the fact that there were no significant differences between pre- and postwash IC_{50} ratios.[13] The fact that no antagonism of morphine (μ-selective) or ethylketazocine (κ-selective) was observed when NTII (1) (100 nM) was incubated (30 min) with the guinea pig ileal longitudinal muscle preparation[14] (GPI) suggests that NTII is a δ-selective, irreversible opioid receptor antagonist.

The irreversible blockage by NTII (30-min incubation) was concentration dependent, as indicated by the increase in the postwash IC_{50} ratio (8.6 ± 0.5 at 100 nM vs 42.3 ± 5.8 at 500 nM) of DADLE in the MVD. Moreover, the irreversible blockage of DADLE increased as a function of time, achieving an IC_{50} ratio of 18 at 60 min (Figure 2).[15]

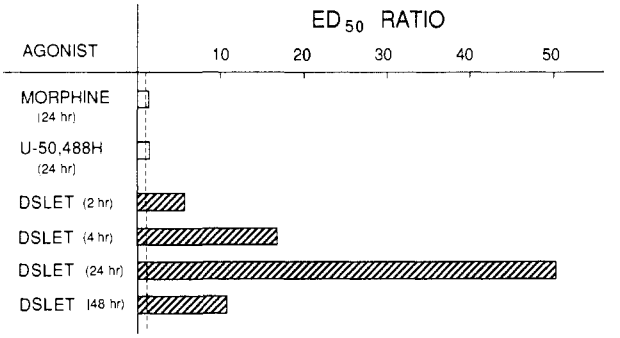

Figure 3. The time course of the antagonist action of NTII toward the antinociceptive effect of DSLET in mice. ED_{50} ratios were calculated by dividing the ED_{50} of the agonist in the presence of NTII by the control ED_{50} of the agonist. The dashed line represents a ratio of 1 (no change).

This time dependence may reflect the rate of covalent association with the δ receptor.

Administration of NTII (10 nmol) to mice by the icv route 24 h prior to testing with the δ-selective agonist Tyr-D-Ser-Gly-Phe-Leu-Thr[11,16] (DSLET), using the abdominal stretch assay,[17] gave an ED_{50} ratio of 52.1 (27.3–106.4). Morphine and the κ agonist *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide[18] (U-50,488H) gave ED_{50} ratios of 1.25 (0.56–3.88) and 3.18 (1.49–13.35), respectively, under these conditions. As illustrated in Figure 3, the time-course of δ receptor antagonism shows that the ED_{50} ratio for DSLET peaked at 24 h and then declined to 10 at 48 h. The in vivo data show that NTII displays a selectivity profile that is similar to those in smooth-muscle preparations, and its antagonist effect may take place through a covalent-binding mechanism.

The in vitro and in vivo data demonstrate that NTII is a potent and selective, nonequilibrium δ opioid receptor antagonist. In this regard, NTII complements δ-selective ligands that are presently available and has the added advantage of being active in vivo.

Acknowledgment. We thank Michael Powers and Mary Schwartz for the biological testing. This work was supported by the National Institute on Drug Abuse.

(9) Cyclization to the indole was carried out under conditions of the Fischer indole synthesis as described in ref 7b. Isothiocyanate 1 was isolated as the hydrated salt ($C_{27}H_{25}O_3S$·HCl·3.5H_2O) C, H, N.

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(13) The postwash procedure involved the following sequence: four washes after incubation with NTII, incubation in 500 nM naltrexone solution for 5 min, followed by 40 washes. Krebs-Ringers was employed in all experiments.

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(15) As a control experiment, there was no significant difference between the IC_{50} values obtained at 30- and 60-min incubation times of the nonelectrophilic ligand NTI (2).

(16) DSLET was administered icv and its control ED_{50} value was 0.005 nmol (0.003–0.007) per mouse.

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Received October 12, 1989