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Rapid communication

[^{3}H]Tyr-D-Ser-Gly-Phe-Leu-Thr: A SPECIFIC PROBE FOR THE δ-OPIATE RECEPTOR SUBTYPE IN BRAIN MEMBRANES

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The occurrence of multiple opiate receptor subtypes, μ (morphine) and δ (enkephalin) in brain (Lord et al., 1977; Chang and Cuatrecasas, 1979) would explain the various pharmacological responses elicited upon central administration of either opiate alkaloids or opioid peptides. μ-Agonists are potent analgesics whereas δ agonists appear to be more efficient in producing epileptic seizures and 'behavioral' responses. Therefore, much work is under way aimed at designing new molecules which, ideally, would display an absolute specificity for either μ- or δ-receptor subtypes in the CNS. Screening for such drugs classically involves bioassays on peripheral organs: the guinea pig ileum (PGI) and the mouse vas deferens (MVD) (Kosterlitz et al., 1980). Until now, the agonist with the most pronounced δ-selectivity in these tests has been Tyr-D-Ala-Gly-Phe-D-Leu (DADLE), a metabolically stable enkephalin analogue. Actually, DADLE is 88 times as potent in inhibiting the electrically evoked contractions of the MVD as those of the GPI (Kosterlitz et al., 1980). However labelled DADLE, even in very low concentrations, shows substantial cross-reactivity with μ receptors in brain membranes (Chang and Cuatrecasas, 1979; Leslie et al., 1980).

Recently, we have reported the synthesis of the hexapeptide Tyr-D-Ser-Gly-Phe-Leu-Thr (DSTLE) which is more than 620 times as potent on the MVD as on the GPI (Gacel et al., 1980). In this preliminary communication, we have compared the opiate receptor binding interactions of [^{3}H]DSTLE with those of [^{3}H]DADLE in the same membrane preparations. All experiments were carried out on a crude membrane fraction (CMF) from whole (minus cerebellum) rat brain. Specific binding was always defined as the portion of total binding which was inhibited in the presence of 10 μM levorphanol. The binding curve of [^{3}H]DADLE at concentrations in the range 0.2–20 nM suggested interaction of this radioligand with two distinct classes of binding sites. The Scatchard plot was curvilinear (fig. 1). The parameters characteristic of DADLE binding to the high affinity site were K_{D1} 2×10^{-9} M and B_{1max} 0.10 pmol/mg of protein and those to the low affinity site $K_{D2} \sim 2 \times 10^{-8}$ M and $B_{2max} \sim 0.20$ pmol/mg of protein in good agreement with previous reports (Chang and Cuatrecasas, 1979; Leslie et al., 1980).

In marked contrast with this, [^{3}H]DSTLE, over the same range of concentrations (0.2–20 nM) appeared to interact with one single class of independent binding sites leading to a linear derived Scatchard plot (fig. 1). The dissociation constant of DSTLE was K_D 2.6 nM and its binding capacity, B_{max} 0.11 pmol/mg of protein. Significantly enough, the maximum number of binding sites for [^{3}H]DSTLE was equal to that of [^{3}H]DADLE high affinity (δ) sites. The δ selectivity of unlabelled DSTLE was also evaluated indirectly using low concentrations of the μ agonist [^{3}H]dihydro-

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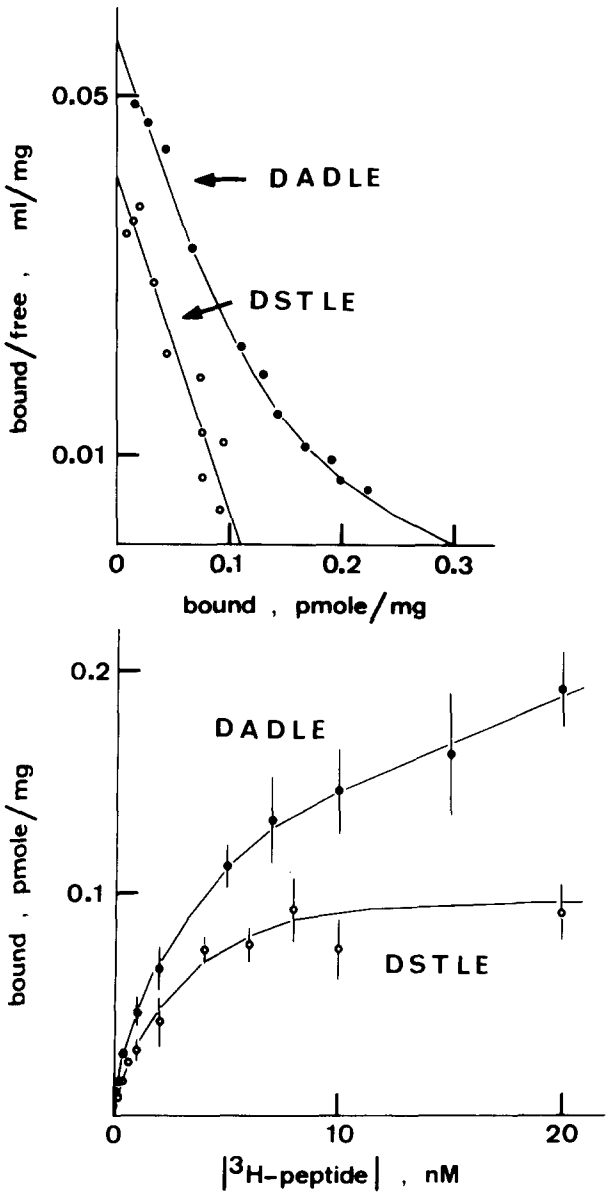

Fig. 1. Saturation isotherms (*bottom*) and Scatchard representations (*top*) of the binding of [^{3}H]DADLE and [^{3}H]DSTLE to a crude membrane fraction from rat brain. Crude membrane fractions from adult Wistar rats (200–250 g) were obtained as described (Chang and Cuatrecasas, 1979). Equilibrium binding experiments were performed at 35°C in Tris·HCl 50 mM, pH 7.4. Each assay mixture (1 ml, in triplicate) contained 0.30–0.35 mg of CMF protein, [D-Ala^2] [tyrosyl-3,5-^{3}H]enkephalin [5-D-leucine] ([^{3}H]DADLE, 42 Ci/mmol, the Radiochemical Center, Amersham) or [D-Ser^2] [tyrosyl-3,5-^{3}H]enkephalin [5-L-leucine, 6-L-threonine] ([^{3}H]DSTLE, 40 Ci/mmol) at the desired concentration without and with 10 μM (final) levorphanol. After a 30 min incubation, the reaction was stopped by rapid cooling and immediate filtering through glass fiber disks (Whatman GF/B). Unbound or loosely bound radioactivity was washed away with two 5 ml portions of ice-cold buffer. The filters were then dried and the radioactivity counted. For [^{3}H]DSTLE the value of specific binding at maximal occupation is 43.2 ± 5.6 of total binding. The total specific radioactivity bound was 2780 ± 215 cpm. On this figure, each value is the mean ± S.D. of the values from independent experiments carried out on six different preparations.

morphine ([^{3}H]DHM) and of [^{3}H]DADLE which exhibits partial specificity for δ-receptors. The inhibition curves of morphine, DADLE and DSTLE were drawn and the potency of each compound was determined from the corresponding linear Hill plots. It was found that morphine, DADLE and DSTLE, were respectively 81, 0.34 and 0.10 times as potent inhibitors of [^{3}H]DHM (0.5 nM) binding as of [^{3}H]DADLE (2.0 nM) binding to the CMF. Thus, the discrimination ratio (Kosterlitz et al., 1976) for DSTLE was one third that for DADLE indicating clearly that DSTLE was a better δ-selective ligand than DADLE. This result is at least qualitatively similar to the one obtained from bioassays on the GPI and on the MVD (Gacel et al., 1980).

In conclusion, at concentrations up to 20 nM, [^{3}H]DSTLE as opposed to [^{3}H]DADLE, interacts with one single class of sites whose maximum number is equal to that of [^{3}H]DADLE high affinity (δ) sites in the rat brain CMF. In other words, DSTLE, as opposed to DADLE exhibits no detectable cross-reactivity with μ-receptor sites in vitro under our experimental conditions. Therefore, [^{3}H]DSTLE shows itself to be the radioligand of choice for quantitative biochemical binding assays for δ-receptor subtypes.

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