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Short communication

Tyrosine-iodination converts the δ-opioid peptide antagonist TIPP to an agonist

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Abstract

The binding properties and pharmacological activities of H-Tyr(3′-I)-Tic-Phe-Phe-OH ([Tyr(3′-I)[1]]TIPP) were studied. Similar to the δ-opioid receptor antagonist H-Tyr-Tic-Phe-Phe-OH (TIPP), [Tyr(3′-I)[1]]TIPP is a selective and potent ligand at δ-opioid receptors. The displacement curve of [^{3}H]diprenorphine binding by [Tyr(3′-I)[1]]TIPP was shifted to the right in the presence of Na^+ and 5′-guanylylimidodiphosphate, suggesting that it acted as a δ-opioid receptor agonist. [Tyr(3′-I)[1]]TIPP also behaved as a full agonist in the mouse vas deferens assay and its effect was both naloxone- and TIPP-reversible. These data show that monoiodination at the 3′-position of the N-terminal tyrosine aromatic ring of TIPP converted it from a potent and selective antagonist to a full agonist at δ-opioid receptors.

Keywords: δ-Opioid receptor; TIPP (H-Tyr-Tic-Phe-Phe-OH); [Tyr(3′-I)[1]]TIPP; Brain, rat; N4TG1 cell; Vas deferens, mouse

1. Introduction

In the last decade, through extensive research in in vitro and in vivo pharmacological models, unequivocal evidence has been established for the existence of at least four types of opioid receptors: μ, δ, κ and ε (Chang, 1984). The multiple receptor classification is mainly based on the selectivity of particular ligands for one receptor type over the others. Attempts to obtain analogues of opioid peptides with high affinity and selectivity for a specific class of receptors were a major goal in the investigations on morphinomimetic peptides (Schiller, 1991). Recently, several laboratories have reported the molecular cloning of the μ- (Chen et al., 1993), δ- (Evans et al., 1992; Kieffer et al., 1992), and κ- (Yasuda et al., 1993) opioid receptors. The availability of the cloned receptors now allows for studies of individual opioid receptor types with regard to pharmacological profile and structure-function analysis.

Many δ-opioid receptor agonists have been discovered and amongst them the cyclic enkephalin analog [D-Pen^2,D-Pen^5]enkephalin (DPDPE) (Mosberg et al., 1983) and the naturally occurring deltorphins (Erspamer et al., 1989) are the most potent and selective δ-opioid receptor peptide agonists. Recently, H-Tyr-Tic-Phe-Phe-OH (TIPP), a potent and highly selective peptide δ-opioid receptor antagonist consisting entirely of aromatic amino acid residues and containing tetrahydro-3-isoquinoline carboxylic acid (Tic) in the 2-position of the peptide sequence, has been described (Schiller et al., 1992a). We report here that monoiodination of TIPP at the 3′-position of the Tyr[1] aromatic ring converted this highly selective peptide δ-opioid receptor antagonist into an agonist.

2. Materials and methods

N4TG1 neuroblastoma cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) calf serum and incubated in a humidified atmosphere with 5% CO_2 at 37° C. Crude membrane preparations from N4TG1 cells or rat brain and guinea pig cerebellum were prepared by homogenization with

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a Polytron PT-20 homogenizer in 50 mM ice-cold Tris · HCl (pH 7.7) containing 50 μg/ml soybean trypsin inhibitor and 10 μg/ml leupeptin (buffer A). The homogenates were centrifuged at 6000 × *g* at 4° C for 15 min to separate the nuclei and mitochondria. The supernatants were centrifuged at 40 000 × *g* at 4° C for 30 min. The pellet consisting of crude membranes was then suspended in buffer A and stored at −80° C until use.

Aliquots of N4TG1 cell membranes (20 μg protein) or brain membranes (500 μg protein) were suspended in binding buffer (Tris · HCl 50 mM, pH 7.7; leupeptin 20 μg/ml; bacitracin 50 μg/ml; thiorphan 20 μg/ml; $MgCl_2$ 5 mM and bovine albumin 0.1%) and incubated with either [^{3}H]Tyr-D-Ala-Gly-*N*-methyl-Phe-Gly-ol ([^{3}H]DAMGO) (specific activity 50.5 Ci/mmol, NEN), [^{3}H]DPDPE (specific activity 46 Ci/mmol, Amersham) and [^{3}H]U69593 (specific activity 47 Ci/mmol, NEN) at 24° C for 60 min or [^{3}H]diprenorphine (specific activity 46 Ci/mmol, Amersham) at 24° C for 90 min. The binding reaction was terminated by adding 2 ml of ice-cold 50 mM Tris · HCl (pH 7.7) buffer and followed by rapid filtration through Whatman GF/C glass filters. The filters were washed twice with 5 ml of ice-cold 50 mM Tris · HCl buffer under vacuum. Nonspecific binding was determined in the presence of 10 μM naloxone. All assays were performed in duplicate; variability of the duplicates was usually less than 10% of the mean value. Competition curve analyses were carried out with the EBDA and LIGAND computer programs (McPherson, 1983).

The mouse vas deferens assay was carried out as reported (DiMaio et al., 1982). A log dose-response curve was determined with [Leu^5]enkephalin as standard for each mouse vas deferens preparation, and IC_{50} values of [Tyr(3′-I)^1]TIPP were normalized according to a published procedure (Waterfield et al., 1979).

[Tyr(3′-I)^1]TIPP was synthesized by the usual solid-phase technique with N^{α}-*t*-butyloxycarbonyl-protected amino acids and with dicyclohexylcarbodiimide/1-hydroxybenzotriazole as coupling agents according to a protocol described by Schiller et al. (1992b). The crude peptide was purified by gel filtration and by reversed-phase chromatography. Homogeneity of the peptide was established by thin layer chromatography (TLC) in two systems and by analytical high performance liquid chromatography (HPLC), and its molecular weight was determined by fast atom bombardment mass spectrometry (molecular ion at *m/e* 761). Analytical characterization: TLC R_f 0.66 (*n*-BuOH/AcOH/H_2O [4/1/5, organic phase]), R_f 0.81 (*n*-BuOH/pyridine/AcOH/H_2O [15/10/3/12]); HPLC K′ = 1.08 (Vidac column [4.6 × 250 mm], 0.1% TFA/CH_3CN 62/38, flow rate 1.2 ml/min, monitored at 280 nm). Naloxone · HCl and DPDPE were purchased from Endo Laboratory (Garden City, NY, USA) and Peninsula Laboratories (Belmont, CA, USA), respectively.

3. Results

The opioid receptor selectivity of [Tyr(3′-I)^1]TIPP was examined by determining its ability to inhibit the binding of selective radioligands to μ- and δ-opioid receptors in rat brain membranes and to κ-opioid receptors in the guinea pig cerebellum. [Tyr(3′-I)^1]TIPP exhibited high affinity for δ-opioid receptors with K_i = 10.3 nM (Fig. 1). It has 10-fold lower δ-opioid receptor affinity than its parent peptide, TIPP, which has a K_i value of 1.1 ± 0.3 nM (n = 3). [Tyr(3′-I)^1]TIPP was much less potent in competing with [^{3}H]DAMGO binding to μ-opioid receptors (Fig. 1) and it did not inhibit [^{3}H]U69593 binding to κ-opioid receptors in guinea pig cerebellum at concentrations up to 25 μM (results not shown). These data indicate that [Tyr(3′-I)^1]TIPP is a potent and selective δ-opioid receptor ligand.

In order to further characterize the binding properties of [Tyr(3′-I)^1]TIPP, binding competition assays based on displacement of [^{3}H]diprenorphine by TIPP, [Tyr(3′-I)^1]TIPP and DPDPE to N4TG1 cell membranes were conducted. These cells contain opioid receptors of the δ type only (Chang et al., 1978). The binding assays were performed under control conditions (Tris · HCl binding buffer) or with the addition of 100 mM Na^+ and 0.1 mM 5′-guanylylimidodiphosphate (Gpp(NH)p). The binding of [^{3}H]diprenorphine to N4TG1 cell membranes was saturable and Scatchard analysis revealed a single high affinity site with K_d = 0.6 nM. The binding of [^{3}H]diprenorphine to this site was not affected by the addition of Na^+ and Gpp(NH)p. In

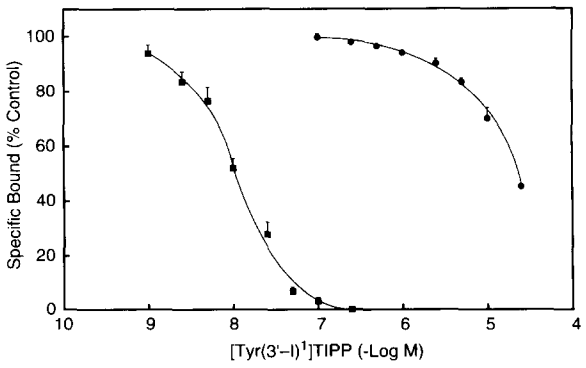

Fig. 1. Competition binding curves of [Tyr(3′-I)^1]TIPP obtained by displacing the δ-opioid receptor ligand [^{3}H]DPDPE (■; 0.5 nM) and the μ-opioid receptor ligand [^{3}H]DAMGO (●; 0.5 nM) from rat brain membrane binding sites. Data shown are mean ± S.E.M. of three separate experiments. The apparent dissociation constants (K_i) are 10.3 ± 1.5 nM and 17.5 ± 2.5 μM for δ- and μ-opioid receptors, respectively.

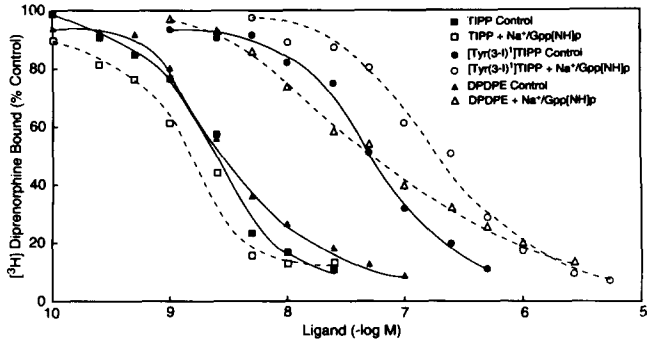

Fig. 2. Na^+ and Gpp(NH)p regulation on TIPP, DPDPE, and [Tyr(3′-I)^1]TIPP competition against [^{3}H]diprenorphine binding. The competition of different ligands against the binding of [^{3}H]diprenorphine (0.2 nM) to N4TG1 cell membranes was determined in the absence (filled symbols) or presence (open symbols) of Na^+ (100 mM) and Gpp(NH)p (0.1 mM) in Tris·HCl binding buffer. Data shown are mean values of three separate determinations.

the presence of Na^+ and Gpp(NH)p, the displacement curve of DPDPE, a δ-opioid receptor agonist, was shifted to the right (Fig. 2), resulting in a 17-fold increase in the IC_{50} value, from 2.5 ± 0.1 to 43.5 ± 11.6 nM. In contrast, the IC_{50} value of TIPP (2.8 ± 0.3 nM) for displacing bound [^{3}H]diprenorphine remained unchanged in the presence of Na^+ and Gpp(NH)p (2.1 ± 0.2 nM). These results showed that the binding affinity of TIPP, a δ-opioid receptor antagonist, was not affected by Na^+ and Gpp(NH)p. Na^+ and Gpp(NH)p also shifted the displacement curve of [Tyr(3′-I)^1]TIPP to the right with the IC_{50} value significantly increased ($P < 0.05$) from 46.0 ± 3.1 to 125.8 ± 17.6 nM.

In the mouse vas deferens assay, [Tyr(3′-I)^1]TIPP behaved as a full agonist with an IC_{50} value of 97.4 ± 8.3 nM. Its potency was about 9 times lower than that of [Leu^5]enkephalin (IC_{50} = 11.4 ± 1.1 nM). The agonist effect of [Tyr(3′-I)^1]TIPP was fully reversed by naloxone (100 nM) and TIPP (100 nM).

4. Discussion

The present study shows that [Tyr(3′-I)^1]TIPP is a potent and selective ligand at δ-opioid receptors with a receptor binding profile similar to that of its parent peptide TIPP. Guanine nucleotides have been shown to affect agonist binding to G-protein coupled receptors by increasing the agonist dissociation rate from the receptor and thus decreasing agonist affinity, whereas antagonists bind with the same high affinity to both states (Chang et al., 1983). Our results showed that the displacement of [^{3}H]diprenorphine binding by TIPP was not influenced by Na^+ and Gpp(NH)p, confirming that TIPP acts as an antagonist at δ-opioid receptors. This observation is in accord with results obtained in the mouse vas deferens assay, where TIPP showed no agonist activity at concentrations up to 10 μM but was a potent antagonist against various δ-opioid receptor agonists (Schiller et al., 1992a). Sodium ion and Gpp(NH)p, however, reduced the binding affinity of [Tyr(3′-I)^1]TIPP and DPDPE to δ-opioid receptors in N4TG1 cell membranes. These data strongly suggest that monoiodination at the 3′-position of the N-terminal amino acid tyrosine of the tetrapeptide TIPP converted it from a selective δ-opioid receptor antagonist to an agonist.

The agonist properties of [Tyr(3′-I)^1]TIPP were confirmed in the mouse vas deferens assay, a functional assay representative for δ-opioid receptor interactions. In this assay, [Tyr(3′-I)^1]TIPP was a full agonist with an IC_{50} of 97.4 nM and, in comparison with [Leu^5]enkephalin, had a potency of about 12%. The effect was both naloxone- and TIPP-reversible, indicating that the agonist effect was mediated by δ-opioid receptors. The potency of [Tyr(3′-I)^1]TIPP observed in the mouse vas deferens assay was somewhat lower than the δ-opioid receptor affinity obtained in the receptor binding assay. The IC_{50} determined in the mouse vas deferens assay did not change in the presence of various protease inhibitors (unpublished results), suggesting that peptide degradation was not a factor. This result suggests that while [Tyr(3′-I)^1]TIPP is a full agonist, it has low efficacy at the δ-opioid receptors in the mouse vas deferens.

In conclusion, these data are the first ones to show that monoiodination at the 3′-position of the N-terminal tyrosine aromatic ring of the opioid tetrapeptide TIPP converted it from a δ-opioid receptor antagonist to a full agonist which retained high affinity for the δ-opioid receptors. This striking observation may be due to the fact that introduction of the bulky and lipophilic iodine substituent may stabilize a conformation which permits receptor activation and which may not be assumed by the parent antagonist peptide TIPP.

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