ELSEVIER

European Journal of Pharmacology 273 (1995) 307–310

ejp

Short communication

Interactions of *erythro*-ifenprodil, *threo*-ifenprodil, *erythro*-iodoifenprodil, and eliprodil with subtypes of σ receptors

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Received 8 December 1994; accepted 13 December 1994

Abstract

Observations of sigma (σ) receptor heterogeneity have prompted interest in identifying ligands for σ receptor subtypes. Selective ligands for the σ-2 are unavailable, but [^{3}H]ifenprodil labels σ-2 sites. Therefore, isomers and analogues of ifenprodil were compared as potential σ-2 ligands. *Threo*-ifenprodil and *erythro*-ifenprodil had high affinity ($K_i \simeq 2$ nM) for σ-2 sites; *erythro*-iodoifenprodil had moderate affinity ($K_i \simeq 46$ nM); eliprodil had lowest affinity ($K_i \simeq 630$ nM). *Threo*-ifenprodil, which has less affinity for α_1-adrenoceptors than *erythro*-ifenprodil, was slightly more selective than *erythro*-ifenprodil for σ-2 sites. These results identify *threo*-ifenprodil as potentially useful for studies of σ-2 receptors.

Keywords: σ Receptor; Ifenprodil; Eliprodil; Subtype; Receptor binding; Brain, rat

1. Introduction

Sigma (σ) receptors in brain have been implicated in diverse actions, including the production of psychosis, antipsychotic activity, locomotor function, and neuroprotection (reviews Ferris et al., 1991; Su, 1993; Walker et al., 1990). Although subtypes of σ receptors (σ-1 and σ-2) have been identified, the functional roles of these subtypes are unclear (Quirion et al., 1992). The (+)-stereoisomers of pentazocine and *N*-allylnormetazocine (SKF 10,047) demonstrate high (nanomolar) affinity for σ-1 sites, but only low (micromolar) affinity for σ-2 sites. Whereas [^{3}H](+)-pentazocine has been used as a radioligand for labeling σ-1 sites (Bowen et al., 1993), selective σ-2 ligands are still unavailable. Recent findings indicate that [^{3}H]endo-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1*H*-benzimidazole-1-carboxamide hydrochloride ([^{3}H]BIMU-1) has over 200-fold greater affinity at σ-2 binding sites than at σ-1 sites (Bonhaus et al., 1993), but this ligand has high affinity for 5-HT$_3$ and 5-HT$_4$ serotonin receptors (Baxter and Clarke, 1992). Therefore, the development of selective σ-2 ligands, particularly for in vivo functional studies, would be of value.

Ifenprodil (*erythro*-ifenprodil) and its analogue eliprodil (SL 82.0715) (Fig. 1) have cytoprotective properties in animal models of brain ischemia (review Carter et al., 1992). It has been suggested that these drugs exert their activity through inhibition at polyamine sites, which positively modulate the NMDA receptor (Carter et al., 1992). In addition to their putative function as NMDA receptor antagonists, these drugs are potent ligands of α_1-adrenoceptors (Chenard et al., 1991) and σ receptors (Contreras et al., 1990). It has been reported recently that simply changing the relative stereochemistry of ifenprodil from the *erythro* to the *threo* diastereomer causes an 8-fold reduction in α_1-adrenergic affinity while enhancing activity at the NMDA receptor 5-fold (Chenard et al., 1991).

The binding of [^{125}I]iodoifenprodil (*erythro* diastereomer) (Fig. 1) to rat cortical membranes is unaf-

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SSDI 0014-2999(94)00763-2

fected by some σ ligands ((+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine (3-PPP), GBR 12909, DTG), suggesting that *erythro*-iodoifenprodil does not interact with σ receptors (Beart et al., 1991). In contrast, [^{3}H]ifenprodil (*erythro* diastereomer) labels σ receptors in rat brain, with selectivity for subtypes (Hashimoto and London, 1993). The rank order of potencies of a series of drugs in inhibiting [^{3}H]ifenprodil binding is highly correlated with their potencies in inhibiting binding at σ-2 but not σ-1 sites (Hashimoto and London, 1993).

The present study was undertaken to examine the interactions of *erythro*-ifenprodil, *threo*-ifenprodil, *erythro*-iodoifenprodil and eliprodil at subtypes of σ receptors in rat brain. The objective was to determine which, if any, of the analogues may have greater affinity and/or selectivity for σ-2 sites, and may therefore be useful as a σ-2 ligand.

2. Materials and methods

Erythro-ifenprodil tartrate and *erythro*-iodoifenprodil (custom synthesis) were purchased from Research Biochemicals Int. (Natick, MA, USA). Eliprodil and *threo*-ifenprodil were obtained from Synthelabo Recherche (L.E.R.S.) (Bagneux, France) and Pfizer (Groton, CT, USA), respectively. Other chemicals were purchased commercially at analytical grade.

Male Fischer-344 rats (250–300 g) were killed by decapitation, and the brains were rapidly removed. The brains were homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer (pH 8.0 at 25°C) with a Kinematica Polytron homogenizer (Lucerne, Switzerland) at setting 5 for 30 s. The homogenate was centrifuged at $48\,000 \times g$ for 10 min (4°C). The resulting pellet was resuspended in the buffer, and was recentrifuged. This procedure was repeated once more. The final pellet was suspended in 20 volumes of 50 mM Tris-HCl buffer (pH 8.0 at 25°C). Binding assays for subtypes of σ receptors were performed using a slight modification of a published method (Bowen et al., 1993). For assays of binding to σ-1 sites, aliquots of crude membranes (approximately 350–400 μg protein) were incubated with [^{3}H](+)-pentazocine (35.3 Ci/mmol, DuPont/NEN, Boston, MA, USA) and 50 mM Tris-HCl buffer (pH 8.0 at 25°C) in a final volume of 0.5 ml for 2 h at 25°C. For assays of binding to σ-2 sites, aliquots of crude membranes (approximately 350–400 μg protein) were incubated with [^{3}H]DTG (37.2 Ci/mmol, DuPont/NEN, Boston, MA, USA), 1 μM (+)-pentazocine, and 50 mM Tris-HCl (pH 8.0 at 25°C) in a final volume of 0.5 ml for 2 h at 25°C. After addition of 4 ml of ice-cold buffer, the membranes were rapidly filtered using a Brandell 48-channel cell harvester (Biochemical Research Laboratory, Gaithersburg, MD, USA) through Whatman GF/B filters pretreated with 0.5% polyethyleneimine for at least 2 h. The filters were washed with three 4-ml aliquots of ice-cold buffer. The radioactivity trapped by the filters was determined by liquid scintillation counting (Beckman LS-3801) at an efficiency of about 50%. Nonspecific binding was estimated in the presence of 10 μM haloperidol. Protein concentrations were measured colorimetrically after reaction with a concentrated dye reagent (Bio-Rad, Richmond, CA, USA), using bovine serum albumin as the standard. Values of the dissociation constant (K_d), of the density of binding sites (B_{max}), and of the inhibitor affinity constant (K_i) were calculated using the EBDA and LIGAND programs (Biosoft, UK), as modified for the IBM personal computer ($K_i = IC_{50}/(1 + [L]/K_d$, where [L] was the concentration of radioligand used and IC_{50} was the concentration that resulted in 50% inhibition of specific binding). Statistical analyses were performed using the F test to determine whether data fit better to a single- or two-site model.

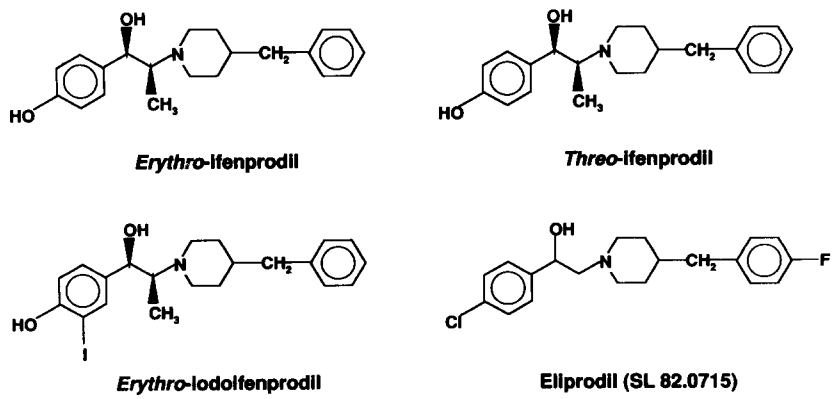

Fig. 1. Chemical structures of *erythro*-ifenprodil, *erythro*-α-(4-hydroxyphenyl)-β-(4-benzylpiperidin-1-yl)-β-methylethanol; *threo*-ifenprodil, *threo*-α-(4-hydroxyphenyl)-β-(4-benzylpiperidin-1-yl)-β-methylethanol; *erythro*-iodoifenprodil, *erythro*-α-(3-iodo-4-hydroxyphenyl)-β-(4-benzylpiperidin-1-yl)-β-methylethanol; and eliprodil (SL 82.0715), (±)-α-(4-chlorophenyl)-4-[(4-fluorophenyl)methyl]-1-piperidineethanol.

3. Results

Scatchard analysis of [^{3}H](+)-pentazocine (0.8–100 nM) binding (to σ-1 sites) to rat brain membranes yielded a K_d of 13.1 ± 4.3 nM and a B_{max} of 831 ± 18 fmol/mg protein (mean ± S.E.M., $n = 3$). Specific binding of [^{3}H](+)-pentazocine was potently inhibited by *erythro*-ifenprodil, *threo*-ifenprodil, *erythro*-iodoifenprodil and eliprodil (Fig. 2). The rank order of potencies of drugs in competing with [^{3}H](+)-pentazocine binding was as follows: *erythro*-ifenprodil (K_i = 13.0 ± 1.0 nM) > *threo*-ifenprodil (K_i = 59.1 ± 3.6 nM) > *erythro*-iodoifenprodil (K_i = 122 ± 13 nM) > eliprodil (K_i = 132 ± 26 nM). Inhibition by these four drugs was monophasic with pseudo-Hill coefficients close to unity.

Scatchard analysis of [^{3}H]DTG (0.8–100 nM) binding (to σ-2 sites in the presence of 1 μM (+)-pentazocine) to rat brain membranes yielded a K_d of 40.3 ± 1.4 nM and a B_{max} of 1240 ± 63 fmol/mg protein (mean ± S.E.M., $n = 3$). Specific binding of [^{3}H]DTG (in the presence of 1 μM (+)-pentazocine) was inhibited potently and biphasically by *erythro*-ifenprodil (K_H = 1.89 nM, K_L = 586 ± 272 nM) and *threo*-ifenprodil (K_H = 2.22 ± 0.40 nM, K_L = 145 ± 107 nM). The pseudo-Hill coefficients for *erythro*-ifenprodil and *threo*-ifenprodil were far less than unity, suggesting that these two drugs interacted with more than one population of sites (Fig. 2). Competition curves for *erythro*-ifenprodil and *threo*-ifenprodil revealed that 79% and 89% of the specific binding, respectively, were to a component that was inhibited with high affinity by these drugs. Inhibition of specific binding of [^{3}H]DTG (in the presence of 1 μM (+)-pentazocine) by *erythro*-iodoifenprodil (K_i = 46.2 ± 5.0 nM) and eliprodil (K_i = 634 ± 72 nM) was monophasic with pseudo-Hill coefficients close to unity.

4. Discussion

The *erythro* and *threo* diastereomers of ifenprodil bind with high affinity to both subtypes of σ receptors. Both diastereomers of ifenprodil show slightly higher

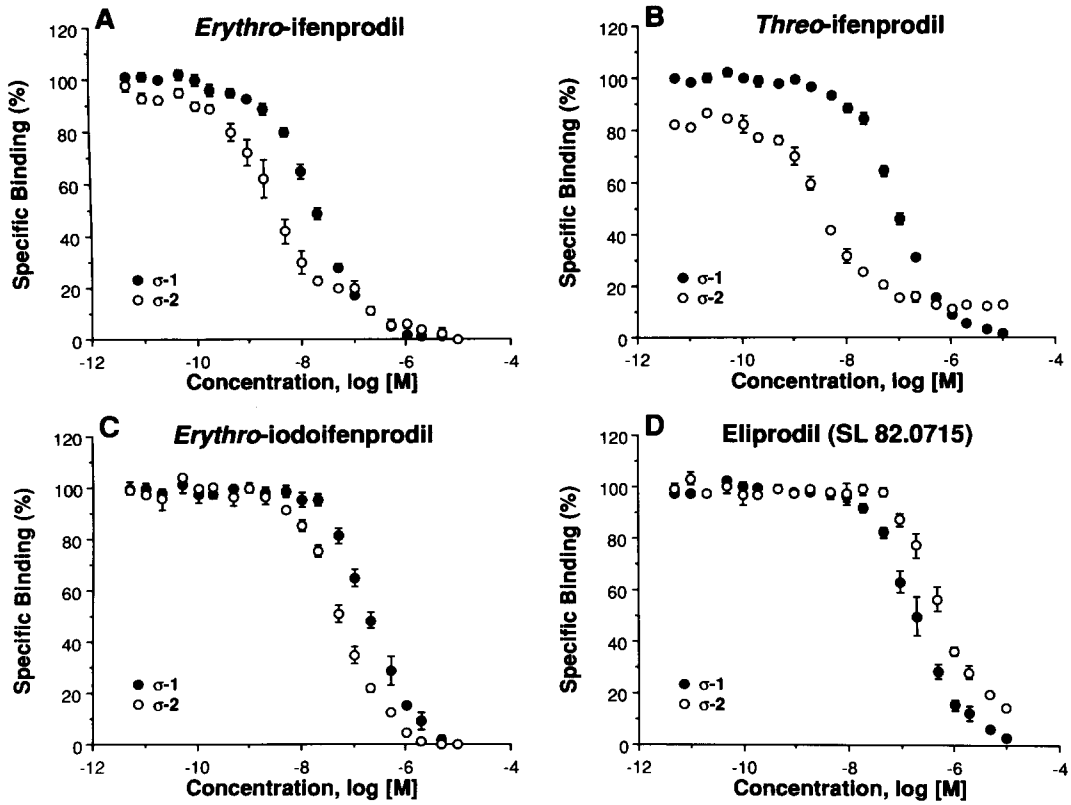

Fig. 2. Competition curves of [^{3}H](+)-pentazocine binding (to σ-1 sites) and [^{3}H]DTG (in the presence of 1 μM (+)-pentazocine) binding (to σ-2 sites) to rat brain membranes. The results are means ± S.E.M. of three separate experiments performed in duplicate. The K_i denotes the affinity constant for binding to a single state of binding sites. When Hill coefficients (n_H) were significantly less than one, competition curves were constructed for a two-site model to obtain K_H and K_L values at high and low affinity sites, respectively. Percentages at each site are given in parentheses. A: *Erythro*-ifenprodil, σ-1 sites: K_i = 13.0 ± 1.0 nM, n_H = 0.94 ± 0.04, σ-2 sites: K_H = 1.89 ± 0.6 nM (79) 0.7%), K_L = 586 ± 272 nM (21) 0.7%), n_H = 0.55 ± 0.04. B: *Threo*-ifenprodil, σ-1 sites: K_i = 59.1 ± 3.6 nM, n_H = 0.92 ± 0.02, σ-2 sites: K_H = 2.22 ± 0.40 nM (89) 2.3%), K_L = 145 ± 107 nM (11) 2.3%), n_H = 0.35 ± 0.01. C: *Erythro*-iodoifenprodil, σ-1 sites: K_i = 122 ± 13 nM, n_H = 1.05 ± 0.05, σ-2 sites: K_i = 46.2 ± 5.0 nM, n_H = 1.01 ± 0.05. D: Eliprodil, σ-1 sites: K_i = 132 ± 26 nM, n_H = 0.90 ± 0.01, σ-2 sites: K_i = 634 ± 72 nM, n_H = 0.79 ± 0.03.

affinity for σ-2 sites than σ-1 sites. The *threo*-diastereomer of ifenprodil is slightly more selective for σ-2 than for σ-1 sites, as the ratio of the K_i values obtained in binding to σ_2 vs. σ_1 sites is about one-fourth that obtained for *erythro*-ifenprodil (0.04 vs. 0.15, respectively).

Beart et al. (1991) have reported that σ ligands (GBR 12909 and DTG) have no significant effects on *erythro*-[^{125}I]iodoifenprodil binding to cortical membranes, suggesting that the bulky iodine atom prevents interaction with σ receptors. However, our data show that *erythro*-iodoifenprodil binds both subtypes of σ receptors with moderate affinities, although the introduction of iodine into *erythro*-ifenprodil decreases affinity for both subtypes of σ receptors as compared with *erythro*-ifenprodil. Furthermore, the introduction of an iodine atom does not impair selectivity for σ-2 sites. Therefore, a σ ligand, such as GBR 12909, should be used to mask σ receptors if *erythro*-[^{125}I]iodoifenprodil is used for binding assays for polyamine-sensitive sites associated with the NMDA receptors, as reported previously (Hashimoto et al., 1994; Schoemaker et al., 1990).

Eliprodil is a less potent ligand for both subtypes of σ receptors than *erythro*- and *threo*-diastereomers of ifenprodil, and the affinity ($K_i = 132$ nM $\pm$ 26) of eliprodil for σ-1 sites is similar to that ($K_i = 122 \pm 13$ nM) of *erythro*-iodoifenprodil. Eliprodil is less potent than the other three compounds at σ-2 sites.

In conclusion, it appears that the stereochemistry of ifenprodil may play a significant role in the selectivity for σ-2 sites. Of the four compounds studied, the *threo* diastereomer of ifenprodil interacted most preferentially with the σ-2 site, but only with 26-fold greater affinity for the σ-2 versus the σ-1 site. Furthermore, the affinity of the *threo* diastereomers of ifenprodil and its analogues for α_1-adrenoceptors is lower than those of the corresponding *erythro* diastereomers (Chenard et al., 1991). This feature of the *threo* diastereomer of ifenprodil renders it potentially more useful as a selective σ-2 ligand.

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