WIN 44,441: A Stereospecific and Long-Acting Narcotic Antagonist

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Abstract: The opiate antagonist WIN 44,441-3 is a potent, stereospecific antagonist of mu, delta, and kappa opiate receptors. This antagonist activity is of long duration (> 4 h) with no agonist activity being observed. It therefore appears that WIN 44,441-3 will be a useful long-acting opiate antagonist for *in vivo* studies.

Introduction

The concept of multiple opiate receptors (1, 2) within the central nervous system (CNS) has been supported by a large amount of behavioral (1–6), neurochemical (7–10), and receptor binding (11–17) data. The major opiate receptor populations appear to be the mu (μ), delta (δ) and kappa (κ) receptors (18–20). In addition, multiple putative endogenous opioid ligands have been isolated from the brain. These include β-endorphin (21, 22), the enkephalins (23, 24), and the dynorphins (25, 26).

The wide distribution of these opioid systems within the CNS suggests that these peptides are involved in the modulation of many sensory inputs and not exclusively noxious sensory information (27–30). Such functional roles for endogenous opiates have led to speculations of a role for these peptides in psychopathology (31). In this regard, the opiate antagonists naloxone and naltrexone have been reported to be effective in reducing hallucinations in a "subpopulation" of schizophrenics (32–35) and in reducing manic symptoms in a "subpopulation" of bipolar depressives (36). Experimental studies with naloxone have also suggested roles for endogenous opioid peptides in (a) the pathophysiology of shock states (37, 38); (b) the hypotensive actions of clonidine (39); (c) the cardiovascular effects of halothane (40); and (d) endocrine function (41).

Such clinical and experimental studies require a potent narcotic antagonist that is devoid of agonist activity, which is long-acting and which is available in active and inactive stereoisomers. Of the available clinical antagonists, naloxone lacks agonist activity but possesses a half-life between 15 and 30 min (42, 43). In contrast, naltrexone is a long-acting antagonist, but possesses significant agonist activity (44). A stereospecific, long-acting, pure narcotic antagonist is therefore still a required entity for long-term clinical and basic research studies. In this report, we would like to describe a candidate for such a role. This is the antagonist WIN 44,441 (Fig. 1) for which both the (−)-isomer (WIN 44,441-3) and (+)-isomer (WIN 44,441-2) are available (45).

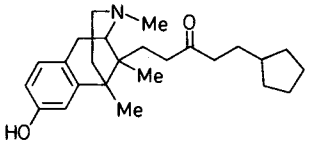

Fig. 1 Structure of WIN 44,441

Methods

Dopamine Metabolism

Male Sprague Dawley rats (Canadian Breeding Labs, St. Constant, Quebec) weighing 180 to 200 g were injected i.p. with morphine and/or WIN 44,441-(2 or 3) or intraventricularly (8) with D-Ala^2-D-Leu^5-enkephalin (DADLE). At various times after drug or peptide administration the rats were sacrificed by microwave irradiation focused on the skull (8) and the striata processed for the gas chromatographic-mass fragmentographic assay (8, 10, 46) of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA).

Receptor Binding

Brain membrane suspensions were prepared from whole brain minus cerebellum as described previously (11, 12). Opiate binding assays were conducted with 1.5 nM ^{3}H-naloxone (Nal; 20 Ci/mmol, NEN); 0.5 nM ^{3}H-dihydromorphine (DHM; 72 Ci/mmol; NEN); 0.3 nM ^{3}H-RX 783006 ((D-Ala^2, NMe-Phe^4, Gly-ol^5)-enkephalin, DAGO; 51 Ci/mmol, Amersham); 1.0 nM ^{3}H-DADLE (39 Ci/mmol, NEN); 2 nM ^{3}H-ethylketazocine (EKC; 15 Ci/mmol; NEN); and 1.0 nM ^{3}H-SKF-10,047 (30 Ci/mmol, NEN). Incubations were performed in 50 mM Tris.HCl, pH 7.7 at 25° C for 60 min (^{3}H-DHM, ^{3}H-DAGO, ^{3}H-DADLE, ^{3}H-SKF-10047) or 120 min at 4° C (^{3}H-Nal ± 120 mM NaCl). For ^{3}H-EKC binding, membranes from guinea pigs were used, with the incubation performed at 4° C for 30 min in 50 mM $K_2HPO_4 \cdot HCl$ (pH 7.4) + 1 mM EDTA · K_2 (11). Incubations were terminated by filtration on GF/B filters with 3×5 ml washes of cold incubation buffer. Specific binding was defined by 10^{-5} M unlabeled ligand or 10^{-5} M levallorphan and represented 70 to 90 % of the bound counts depending upon the labeled ligand. IC_{50} values were determined from 10 to 15 unlabeled drug concentrations and a

Table I. Relative Affinities of Naloxone and WIN 44,441 for Different Opiate Receptors

Ligand	IC_{50}(nM)* Naloxone	WIN 44,441-3	WIN 44,441-2
^{3}H-Naloxone (−Na)	2.0	2.2	1400
(Na ratio)	1.0	1.0	–
^{3}H-DHM	2.2	0.90	1800
^{3}H-DAGO	4.9	0.11	>2000
^{3}H-DADLE	33	0.18	>1500
^{3}H-EKC	12.7	1.3	>1200
^{3}H-SKF 10,047	0.97	0.21	1400

* mean of 2–3 experiments (SD < 10%).

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Table II. Dose Response of WIN 44,441-3 Antagonism of Morphine- and DADLE- dependent increases in striatal DOPAC and HVA[1]

Group (mg/kg, i.p.)	DOPAC (pmol/mg protein)	HVA (pmol/mg protein)
Control	68 ± 3	60 ± 2
Morphine (16)	122 ± 4[2]	81 ± 2[2]
+ WIN 44,441-3 (0.02)	126 ± 3[2]	81 ± 2[2]
(0.2)	88 ± 4[2]	72 ± 2[2]
(2.0)	68 ± 3	62 ± 3
(4.0)	66 ± 4	59 ± 4
+ WIN 44,441-2 (4.0)	128 ± 6[2]	85 ± 3[2]
DADLE [10 μg IVt.]	116 ± 3[2]	100 ± 4[2]
+ WIN 44,441-3 (0.02)	102 ± 2[2]	92 ± 3[2]
(0.2)	85 ± 2[2]	75 ± 3[2]
(2.0)	70 ± 3	65 ± 2
WIN 44,441-3 (0.2)	70 ± 3	61 ± 2
(4.0)	69 ± 4	58 ± 3

[1] 60 min; antagonist was a 6 min pretreatment.
[2] $p < 0.05$. (mean ± SE; N = 7–10).

Table III. Duration of narcotic antagonist activity of WIN 44, 441-3

Group (mg/kg)	Antagonist Pretreatment Time (min)	DOPAC (pmol/mg protein)	HVA (pmol/mg protein)
Control	–	72 ± 4	63 ± 5
Morphine (16)	–	130 ± 6[1]	84 ± 4[1]
+ WIN 44,441-3 (4)	6	74 ± 3	66 ± 4
	60	79 ± 5	64 ± 3
	120	74 ± 2	64 ± 2
	180	79 ± 3	69 ± 3
	240	88 ± 2[1]	73 ± 2
+ naloxone (4)	6	73 ± 3	65 ± 6
	60	121 ± 4[1]	83 ± 3[1]
	120	122 ± 5[1]	80 ± 6[1]

[1] $p < 0.05$ (mean ± SE; N = 7–10); 60 min after morphine

linear fit of the data with probit analysis. Protein assays and statistics (Dunnett's t-test) were performed as previously described (7, 8).

Results

Receptor Binding Profile (Table I)

WIN 44,441-3 was equipotent with naloxone in displacing ^{3}H-naloxone binding and had a sodium ratio of 1.0, indicative of an opiate antagonist. WIN 44,441-2 was 636 times less potent than the active isomer. Similarly, WIN 44,441-3 demonstrated stereoselective displacement of the μ agonists ^{3}H-DHM and ^{3}H-DAGO, the δ agonist ^{3}H-DADLE, and the κ agonists ^{3}H-EKC and ^{3}H-SKF 10047 with affinities greater than those of naloxone. Tested against the δ selective peptide ^{3}H-DADLE, WIN 44,441-3 was considerably more potent than naloxone.

DA Metabolism (Tables II and III)

The μ agonist morphine and the δ agonist DADLE both elevated striatal DA metabolites. These actions were reversed by WIN 44,441-3. The approximate AD_{50} was 0.2 mg/kg and the duration of action greater than 4 h; contrasting with the short duration of antagonism observed with naloxone (Table III). No agonist activity was observed and DA steady-state levels were unperturbed by all treatments.

Discussion

Initial studies (45) with the racemate of WIN 44,441 indicated potent μ antagonism in the rat tail flick assay with no agonist activity in either the tail flick or intracarotid bradykinin assays. Similarly, the racemate was a pure antagonist in the isolated guinea pig ileum with a potency twice that of naloxone in antagonizing the μ agonist normorphine (45). The (−)-isomer also has been shown to be active against Leu-enkephalin in the isolated mouse vas deferens (47). *In vitro* the (−)-isomer has been shown to be equipotent in displacing ^{3}H-naloxone and ^{3}H-bremazocine receptor binding (48). Similarly, our *in vitro* data indicated high affinities for μ, δ, and κ receptors with WIN 44,441-3. In contrast, the (+)-isomer, WIN 44,441-2 possessed extremely low affinities for all 3 opiate receptor populations.

In vivo, WIN 44,441 has been used to demonstrate stereospecific antagonism of hemorrhagic shock in cats (49). Our analysis of μ- and δ-dependent increases in striatal DA metabolism also demonstrated potent stereospecific reversal of these actions with WIN 44,441 possessing equivalent potencies for antagonizing both μ and δ agonists. In addition, our data indicate that this antagonism is of significantly greater duration of action than that observed with naloxone (8). In this *in vivo* assay system, no agonist activity was evident. The observed antagonism of both μ and δ agonists in this assay also represents a reliable test since these are independent receptor populations regulating nigrostriatal dopaminergic neurons (50).

In summary, WIN 44,441-3 appears to be a potent, stereospecific, and long-acting opiate antagonist. This antagonist also possesses a broad spectrum of activity against μ, δ, and κ receptors and is devoid of agonist activity.

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