Drug Development Research 3:253–259 (1983)

A Comparison of Naloxone and Naltrexone in Laboratory Tests Predictive of Antipsychotic Potential

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ABSTRACT

Malick, J.B., R.L. Herman, and J.M. Goldstein: A comparison of naloxone and naltrexone in laboratory tests predictive of antipsychotic potential. Drug Dev. Res. **3**:253–259, 1983.

Naloxone and naltrexone were compared neuropharmacologically, especially in several rodent models considered to be predictive of antipsychotic activity, since narcotic antagonists have been reported to be effective in the treatment of schizophrenia. Naltrexone was found to be a potent antagonist of amphetamine-induced aggregate toxicity in mice; this was in marked contrast to naloxone, which enhanced amphetamine's toxicity. Both naloxone and naltrexone exhibited an unusual profile of activity in the apomorphine-induced stereotypy test in mice in that they antagonized the chewing behavior but failed to antagonize the rearing; this is in contrast to the activity of standard neurolepitcs (e.g., haloperidol, clozapine) which antagonize both behaviors induced by apomorphine. Neither of the narcotic antagonists exhibited any activity in several other tests predictive of antipsychotic activity. Naltrexone was a potent inhibitor of spontaneous locomotor activity in mice; naloxone, although much weaker, also antagonized motor activity. Thus, endorphins may play a role in the modulation of exploratory or locomotor activity. The marked difference in activity between the two antagonists in the amphetamine aggregate toxicity study may indicate a significant neuropharmacological difference between these agents; i.e., naltrexone may be much more than merely a more potent naloxone. Furthermore, naltrexone's potent activity as an antagonist of amphetamine-induced aggregate toxicity in mice may be predictive of a better therapeutic effect in schizophrenia than has been observed with naloxone.

Key words: naloxone, naltrexone, haloperidol, clozapine, conditioned avoidance

Received final version January 6, 1983; accepted February 1, 1983.

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INTRODUCTION

The discovery of the endogenous opiatelike peptides (endorphins) [Hughes et al., 1975] has triggered a wealth of research, the goal of which is to discover the role of such peptides in the control of brain function and behavior. The endorphins may be neurotransmitters or modulators of neuronal activity and, as such, may play an important role in the regulation of emotional behavior.

In animals, β-endorphin (c-fragment of β-lipotropin; β-LPH_{61-91}) produced a marked, long-lasting state of muscular rigidity and immobility that overtly resembled a catatonic state and which was described as being "reminiscent of some aspects of schizophrenia" [Bloom et al., 1976]; this syndrome was rapidly antagonized by naloxone, a narcotic antagonist.

In man, hemodialysis has been reported to produce a dramatic, long-lasting improvement in chronic schizophrenics [Wagemaker and Cade, 1977]; the substance being removed from the patient's bloodstream was believed to be a polypeptide which was not removed by the kidney [Wagemaker, 1977]. A subsequent report [Palmour et al., 1979] indicated that an endorphinlike compound had been isolated from the dialysate of the patients that improved following dialysis. This material has been tentatively identified as [Leu^5]-β-endorphin, a compound which has not been observed in normal individuals and thus may be an abnormal endorphin associated with the disease state. These findings are very preliminary, but if they are confirmed in other laboratories, they will support the hypothesis that the endorphins play a major role in mental disease processes.

Several investigators have investigated the potential therapeutic value of drugs which modify the activity of endorphin systems. Terenius and co-workers [1976] observed elevated endorphin levels in the cerebrospinal fluid of chronic psychotic patients; in addition, they demonstrated a significant decrease in endorphin levels following successful treatment with clozapine, an antipsychotic drug. Gunne and associates [1977] were the first to demonstrate that naloxone could significantly ameliorate the auditory hallucinations in some schizophrenics. Subsequently, several trials of naloxone in schizophrenia have been performed and the results have been both encouraging [Emrich et al., 1977; Orr and Oppenheimer, 1978; Watson et al., 1978] and negative [Davis et al., 1977; Kurland et al., 1977; Volavka et al., 1977]. Naltrexone, an orally-effective, long-acting narcotic antagonist with a minimum of agonist activity [Martin et al., 1973], has not exhibited antipsychotic activity in the majority of studies conducted to date [Gitlin and Rosenblatt, 1978; Mielke and Gallant, 1977; Simpson et al., 1977], although only very small numbers of patients have been studied. In a study conducted by Ragheb and associates [1980], naltrexone was found to benefit two of five newly admitted chronic schizophrenics. Not enough studies have been performed with naltrexone to fully assess its antipsychotic potential.

The present studies were designed to evaluate the comparative pharmacological profiles of naloxone and naltrexone, primarily in rodent models that have been used routinely to predict potential antipsychotic drug activity.

METHODS

Male Wistar rats (Hilltop Laboratories, Scottdale, PA) and male albino mice (Hilltop Laboratories) were used throughout these studies. All animals were maintained at constant temperature (21–23°C), on a 10/14-hr light/dark cycle and received food and water ad libitum. All drugs were dissolved or suspended in an HPMC vehicle (0.1% TWEEN® 80, 0.5% hydroxyproplymethylcellulose in 0.9% NaCl). The drugs used in these studies were naloxone hydrochloride and naltrexone hydrochloride (Endo Laboratories, Inc.), haloperidol (McNeil Laboratories, Inc.), apomorphine hydrochloride (Merck and Co.), d-amphetamine sulfate (Sigma Chemical) and clozapine (Sandoz Pharmaceuticals).

Amphetamine Aggregate Toxicity in Mice

Groups of 18 mice (18–22 g) were used in these studies. Drug or HPMC vehicle was administered intraperitoneally (i.p.) 60 min prior to a subcutaneous (s.c.) injection of d-amphetamine (30 mg/kg); this dose of amphetamine usually produces approximately 50% lethality in grouped

mice, thus permitting the simultaneous observation of either potentiation or antagonism by the test compound. Mice were housed six per cage until dosed with amphetamine, at which time they were transferred to plexiglass cubicles (3 × 4 1/2 × 4 inches) and grouped three per cubicle. The number of deaths were recorded in all groups 4 hr postamphetamine and were expressed as percent change from appropriate vehicle-treated controls.

Mouse Apomorphine-Induced Stereotypy

Mice (22–25 g) were pretreated i.p. with either drug or placebo 30 min prior to apomorphine administration (20 mg/kg, s.c.). This dose of apomorphine always caused 100% of vehicle-treated controls to engage in stereotyped behavior (i.e., rearing and chewing). Mice were placed into small Plexiglass cubicles and observed continually for 60 min postapomorphine (five consecutive 12-min observation periods) and the presence or absence of both rearing and chewing were recorded for each mouse. Results are expressed as percent inhibition of apomorphine-induced stereotyped behavior as compared to same-day vehicle-treated controls.

Rat Conditioned Avoidance

Male rats (400–500 g when tested) were trained to press a lever to avoid an electric footshock according to the following paradigm. A conditioned stimulus (CS; consisting of a cue light, a tone, and the extension of a retractable lever) was presented for 20 sec; if a lever press was made during this time period (an avoidance response), the CS was terminated and a 30-sec intertrial interval was initiated. If the animal failed to respond during the first 20 sec of the trial, a 0.7-mA scrambled footshock was presented for a maximum of 30 sec; a lever press during this second segment (an escape response) terminated the shock and the CS and once again initiated a 30-sec intertrial interval. If a rat failed to respond during either segment of a trial, a "nonescape" was recorded. Each session consisted of a total of 60 such trials. Drug or HPMC was administered orally (p.o.) 60 min prior to testing according to a double-crossover dosing matrix. All rats were tested each day of the work week and received drug on Tuesday and Friday and vehicle on Monday, Wednesday, and Thursday. All experimental programming and data recording were performed by an INTERACT computer system.

Rat Amphetamine-Induced Stereotypy

Male rats (175–200 g) were used in these studies. Drugs were administered orally 60 min prior to amphetamine (10 mg/kg, s.c.); this dose of amphetamine caused 100% of control (placebo-treated) rats to engage in stereotyped behavior (e.g., chewing, side-to-side head movements). Rats were housed individually and scored on an all-or-none basis (i.e., presence or absence of stereotypy) for a total of 1 hr postamphetamine (each rat was scored during each of four consecutive 15-min test segments). Results are expressed as percent reduction in stereotyped movements in drug-treated as compared to vehicle-treated controls.

Rat Apomorphine-Induced Stereotypy

Male rats (150–170 g) were treated with either vehicle (HPMC), naltrexone, naloxone, haloperidol, or clozapine i.p. 30 min prior to the administration of apomorphine (0.3 mg/kg, s.c.). Rats were housed individually and observed for signs of stereotyped behavior (i.e., sniffing and head-searching) at 5, 10, and 15 min postapomorphine. The data are expressed as percent of animals exhibiting stereotypy at each test interval.

Motor Activity Studies

Male mice (18–25 g) were treated with either vehicle, naloxone, or naltrexone s.c. 15 min prior to testing for spontaneous motor activity in an Animex activity monitor (Columbus Instruments, Columbus, OH). Mice were grouped three per chamber and total activity counts were recorded for 30 min. The results were statistically evaluated by comparing the drug-treated groups to the appropriate vehicle-treated control using the Student's t-test.

TABLE 1. Effects of Naltrexone, Naloxone, Clozapine, and Haloperidol on Amphetamine-Induced Aggregate Toxicity in Mice

Drug	Dose (mg/kg, i.p.)	N[a]	% Change in lethality[b]
Naltrexone	3	18	↓ 12
	6	18	↓ 64
	10	36	↓ 80
	17	18	↓ 91
	30	36	↓ 44
	100	18	↑ 12
Naloxone	10	18	↑ 66
	25	18	↑ 44
	50	18	↑ 54
	100	18	↑ 54
Haloperidol	0.03	18	↓ 10
	0.1	18	↓ 64
	0.3	18	↓ 91
	0.75	18	↓ 100
Clozapine	2.5	18	↓ 8
	5	36	↓ 33
	10	36	↓ 57
	30	18	↓ 90

[a]Number of mice tested.
[b]Compared to vehicle-treated control group.

RESULTS

Naltrexone produced a dose-related antagonism of amphetamine-induced aggregate toxicity in mice between 3 and 17 mg/kg, p.o.; however, the dose-response curve was bell-shaped (Table 1). The minimal effective dose (MED; i.e., the lowest dose inhibiting toxicity by 50% or more) for naltrexone was 6 mg/kg, p.o., as compared to 0.1 and 10 mg/kg, p.o., for haloperidol and clozapine, respectively. In contrast, naloxone potentiated amphetamine-induced toxicity at all doses (Table 1).

In the apomorphine-induced stereotypy test in mice, both naloxone and naltrexone antagonized the chewing but not the rearing induced by apomorphine (Table 2). Haloperidol and clozapine both produced a dose-related inhibition of both apomorphine-induced rearing and chewing; however, clozapine appeared to be more potent as a antagonist of rearing as opposed to its antagonism for the chewing behavior (Table 2).

Although they were tested over a wide range of doses, both naloxone (10–100 mg/kg, i.p.) and naltrexone (30–200 mg/kg, p.o.) failed to significantly alter conditioned avoidance responding in rats. As expected, both of the standard reference antipsychotics produced a dose-related inhibition of avoidance responding; the minimal effective doses for haloperidol and clozapine were 0.5 and 20 mg/kg, p.o., respectively.

Both naloxone (10–100 mg/kg, i.p.; 10–200 mg/kg, p.o.) and naltrexone (3–200 mg/kg, p.o.) failed to exhibit any antagonism of amphetamine-induced stereotypical behavior in rats (data not shown). Reference antipsychotics significantly antagonize amphetamine-induced stereotypy, although clozapine was very weak; the minimal effective doses for haloperidol and clozapine were 0.1 and 200 mg/kg, p.o., respectively.

In the apomorphine-induced stereotypy test in rats, neither naloxone (1–100 mg/kg, i.p.) nor naltrexone (1–30 mg/kg, i.p.) exhibited any inhibitory activity. The reference antipsychotics, haloperidol and clozapine, significantly antagonized the stereotyped movements at 1 and 60 mg/kg, i.p., respectively.

TABLE 2. Effects of Naltrexone, Naloxone, Clozapine, and Haloperidol on Apomorphine-Induced Stereotypy in Mice

Drug	Dose (mg/kg, i.p.)	N[a]	% Antagonism of stereotypy	
			Rearing	Chewing
HPMC Control	—	20	0.0	0.0
Naltrexone	1	10	0.0	0.0
	3	10	0.0	0.0
	10	15	0.0	0.0
	17	10	0.0	44.8
	30	10	0.0	74.8
	60	10	0.0	88.0
Naloxone	1	10	0.0	0.0
	3	15	0.0	20.3
	10	10	0.0	76.0
	100	10	0.0	100.0
Haloperidol	1	5	0.0	0.0
	2	25	79.2	55.2
	4	25	97.6	99.2
Clozapine	10	15	8.0	0.0
	20	25	62.4	28.0
	40	10	78.0	30.0
	80	10	100.0	56.0

[a]Number of mice tested.

TABLE 3. Effects of Naloxone and Naltrexone on Spontaneous Motor Activity

Treatment	Dose (mg/kg, s.c.)	N[a]	Motor activity (counts/30 min ± SEM)[b]	P-value[c]
Vehicle	—	9	1,069 ± 144	—
Naloxone	10	9	960 ± 90	NS[d]
	20	9	886 ± 140	NS
	40	9	616 ± 129	<0.05
Vehicle	—	10	1,473 ± 137	—
Naltrexone	0.5	10	1,110 ± 114	NS
	1	10	1,034 ± 123	<0.05
	2	10	979 ± 110	<0.05
	4	10	723 ± 87	<0.001

[a]Number of groups of mice tested; 3 mice/group.
[b]Total counts ± SEM.
[c]Comparison of drug-treated to appropriate vehicle control via Student's t-test.
[d]NS = Not significant.

In the motor activity studies in mice, both naltrexone and naloxone produced dose-related inhibition of spontaneous locomotor activity (Table 3). Naltrexone appeared to be considerably more potent than naloxone as an inhibitor of locomotor responses; although the data does not permit statistical analysis for potency differences, there was an approximately 40-fold difference in the minimal effective doses for producing statistically significant decreases in locomotion between the two antagonists (Table 3).

DISCUSSION

Naltrexone was a potent antagonist of amphetamine-induced aggregate toxicity in mice; activity in this model is generally considered to be predictive of antipsychotic potential since all known neuroleptic drugs, with the possible exception of sulpiride, exhibit activity in this test. In contrast to naltrexone, naloxone failed to antagonize amphetamine aggregate toxicity and, in fact, it potentiated the toxicity at all doses although the effect was not dose-related.

Both naloxone and naltrexone significantly antagonized the chewing behavior induced by apomorphine in mice, although they did not alter the rearing produced by apomorphine. This unusual activity profile is not characteristic of either typical (e.g., haloperidol) or atypical (e.g., clozapine) antipsychotics which antagonize both the rearing and chewing induced by apomorphine in mice. Naloxone and naltrexone both failed to exhibit any activity in any of the other tests (i.e., conditioned avoidance, amphetamine- and apomorphine-induced stereotypy in rats) considered to be predictive of antipsychotic potential.

Thus, as a result of these studies in a battery of tests used to search for potential antipsychotics, naloxone would not be expected to exhibit significant antipsychotic potential in man. However, although only apparently small differences in their profiles existed, the activity of naltrexone in the amphetamine-induced aggregate toxicity test may be predictive of better antipsychotic therapeutic potential in man.

The motor activity studies were performed since naltrexone has been reported to be a potent, specific (i.e., it inhibited locomotion at doses which did not produce ataxia) antagonist of exploratory behavior in rats [Katz, 1979]. The present study confirms this activity with naltrexone in another species, the mouse, and extends it by showing that naloxone, although much weaker, exhibits the same activity. Thus, endorphins may play a role in the control or modulation of locomotor or exploratory behavior.

Dettmar and co-workers [Dettmar et al., 1978] have reported that naloxone antagonized the increase in spontaneous locomotor activity produced by amphetamine in mice and the ipsilateral turning induced by amphetamine in rats lesioned unilaterally with 6-hydroxydopamine in the substantia nigra. As a result of their studies [Dettmar et al., 1978] and those of others [Cox et al., 1976; Harris et al., 1977; Henderson and Westkaemper, 1975; Holtzman, 1976], they proposed that naloxone influenced dopaminergic neurotransmission, perhaps via antagonism of opiate receptors located on presynaptic dopaminergic neurons. It is obvious that the narcotic antagonists cannot be occupying dopamine receptors since they do not antagonize all of the effects produced by amphetamine and apomorphine; in addition, in our laboratories [unpublished results], naltrexone failed to exhibit any activity in the ^{3}[H]-spiroperidol binding assay, nor did it alter the rate of production of the dopamine metabolite, homovanillic acid (HVA).

The difference in activity in the amphetamine aggregate toxicity study between naloxone and naltrexone may indicate a significant pharmacological difference between these drugs; naltrexone may be much more than merely a more potent naloxone. Further neuropharmacological studies will be necessary to clarify the nature of the difference between these two agents.

Several lines of evidence from the clinic suggest that enhanced endorphinergic activity may be the etiologic basis for some forms of schizophrenia [Wagemaker, 1977; Palmour et al., 1979; Terenius et al., 1976; Ragheb et al., 1980]. Although the results with naloxone have been somewhat disappointing in that they are inconsistent, the results of the present study suggest that naltrexone might be a better candidate for study in schizophrenia. Thus far, only very small numbers of schizophrenic patients have been treated with naltrexone and the results have been mixed [Gitlin and Rosenblatt, 1978; Mielke and Gallant, 1977; Simpson et al., 1977; Ragheb et al., 1980]; however, further clinical trials appear warranted.

ACKNOWLEDGMENTS

The authors are grateful for the technical expertise provided by Linda Knobloch, Chris Tyler, Bertina Duncan, and Constance Martin. The authors also wish to thank the many pharmaceutical companies who generously supplied us with samples of the drugs used in these studies.

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