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ORIGINAL INVESTIGATION

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Naloxone blocks the antianxiety but not the motor effects of benzodiazepines and pentobarbital: experimental studies and literature review

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Abstract The role of opioid systems in the anticonflict effect of chlordiazepoxide, diazepam and pentobarbital was evaluated with a modified Vogel procedure. First, morphine, ineffective by itself, was combined with subeffective or marginally effective doses of the benzodiazepines in order to detect possible potentiation. However, the combined treatment reduced licking in the Vogel procedure as well as in a licking test where no shock was administered. Several doses of the benzodiazepines and pentobarbital were then administered in combination with several doses of the opiate antagonist naloxone. A dose-dependent inhibition of anticonflict effect was obtained. In an additional experiment, it was shown that naloxone blocked the effects of diazepam in the elevated plus-maze procedure. Motor deficiencies, as evaluated with a rotarod test, produced by the benzodiazepines and pentobarbital could not be antagonized by naloxone. It is concluded that opioids are important for the anticonflict but not for the motor effects of these drugs. An analysis of published studies concerning the interaction of opioids and benzodiazepines in several procedures supposed to reflect anxiolytic effects shows that the inhibition obtained with naloxone is reliable and not procedure specific. The mechanisms by which opiate antagonists produce this inhibition of anticonflict activity are not known. It is tentatively suggested that opioid activation associated with stress may be a necessary component of anxiolysis.

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Key words Benzodiazepines · Pentobarbital · Opioids · Vogel procedure · Anxiety · Motor deficiencies

Introduction

Despite the spectacular progress in the molecular biology and pharmacology of benzodiazepine receptors (Doble and Martin 1992; Giusti and Arban 1993), much behavioral data concerning the actions of benzodiazepines remains unexplained. For example, it has been reported that several effects of benzodiazepines are blocked by opiate antagonists (see Millan and Duka 1981 for review). Anticonflict effects of diazepam and chlordiazepoxide are antagonized by naloxone in the Geller-Seifter or Vogel procedures (Billingsley and Kubena 1978; Soubrié et al. 1980; Duka et al. 1981; Koob et al. 1980). Stimulatory actions of diazepam on food intake in a stressful environment have also been blocked by naloxone (Soubrié et al. 1980). Furthermore, the enhanced intake of food and water in familiar situations produced by several benzodiazepines can be blocked by naloxone, independently of whether sated or deprived subjects are used (reviewed in Cooper 1983). Finally, benzodiazepine-facilitated intracranial self-stimulation (Lorens and Sainati 1978) and conditioned place preference produced by diazepam (Spyraki et al. 1985) are blocked by naloxone.

Some biochemical data appear to support the hypothesis that endogenous opioids may be related to benzodiazepine actions. Benzodiazepines have been found to modulate enkephalin release (Duka et al. 1979; Wüster et al. 1980; Harsin et al. 1982) and this effect is blocked by naloxone (Duka et al. 1980). Moreover, systemic administration of morphine and intracerebroventricular infusion of β-endorphin enhance benzodiazepine binding (Lopez et al. 1990; Gomar et al. 1993b) while naloxone reduces it (Gomar et al. 1993a). The mechanisms behind these effects are not clear.

However, recent data have challenged the hypothesis that opiate antagonists efficiently block the effects of benzodiazepines. Cannizaro et al. (1987) and Tripp and McNaughton (1991) found naloxone unable to inhibit the effects of chlordiazepoxide in modified Geller-Seifter procedures, and the latter authors obtained similar results with a successive discrimination procedure (Tripp and McNaughton 1987). This coincides with an earlier report (Herling 1983). Furthermore, naloxone has been reported to reduce intake of food and water by itself in both sated and deprived animals (reviewed in Levine et al. 1985; Reid 1985). Similarly, naloxone reduces intracranial self-stimulation in a dose dependent way in the absence of other drug treatment (Schaefer 1988). This makes it difficult to interpret the proposed antagonism of benzodiazepine-enhanced eating, drinking and self stimulation. There is, in addition, a report in which naloxone was found to be unable to reduce diazepam-induced hyperphagia in a stressful environment but effectively antagonized the hyperphagia observed in a familiar environment (Britton et al. 1981). The authors proposed that the primary effect of naloxone is on consummatory behavior rather than on anticonflict actions.

Most of the studies mentioned above employed only one dose of the benzodiazepine and one dose of naloxone. Occasionally, two doses of each were used. It seems, then, that the contradictory findings may be partly explained by discrepancies in doses. One purpose of the present studies was, therefore, to evaluate the ability of several doses of naloxone to block the anticonflict effects of several doses of benzodiazepines in the Vogel procedure. Furthermore, the interaction between diazepam and naloxone was analyzed in the elevated plus-maze (Pellow et al. 1985). It was considered important to use a procedure where no painful stimulation is employed, because of the complex interplay between benzodiazepines and opiates in the mediation of analgesia (Maier 1990; Harris and Westbrook 1994).

The supposed interaction between benzodiazepines and opiates does not seem to apply to other drugs acting at the GABA/benzodiazepine/barbiturate/steroid receptor. In studies where naloxone blocked the effects of benzodiazepines on conflict behavior and on food and water intake, the effects of barbiturates were not blocked (Billingsley and Kubena 1978; Cooper and McGivern 1983; Naruse et al. 1989). The second purpose of the present studies was to determine whether naloxone could block the anticonflict effect of pentobarbital in the Vogel procedure.

If the opiate antagonist naloxone blocks the anticonflict effects, it could be supposed that opiates should be anxiolytic. Experimental evidence for this supposition is weak, however (reviewed by Pollard and Howard 1990). Nevertheless, clinical studies have shown a substantial prevalence of benzodiazepine use among opiate addicts (Brown and Chaitkin 1981; Darke et al. 1993) and methadone maintenance patients report that diazepam enhances the effect of methadone (Kleber and Gold 1978; Stitzer et al. 1981). Even if opiates do not display anxiolytic properties by themselves, it is possible that they may potentiate or be potentiated by benzodiazepines. This was also evaluated in the present experiments.

Most benzodiazepines and barbiturates produce motor deficiencies in doses somewhat larger than those required for anticonflict effects (Ågmo and Fernandez 1991; Ågmo et al. 1991). The last purpose of the present studies was therefore to evaluate the capacity of naloxone to block the motor incoordination produced by benzodiazepines and pentobarbital.

Materials and methods

Subjects

Male Wistar rats (250–350 g) from a local colony were housed under a natural light/dark cycle at an ambient temperature of 22 ± 1° C. They were kept five per cage, and given commercial rat pellets ad lib. Tap water was freely available until 24 h before conflict experiments. For the plus-maze experiments, male Wistar rats were puchased from Janvier, Le Genest Saint Isle, France. These rats were maintained under a 12/12-h light/dark cycle, two per cage.

Apparatus and procedure

The lickometer has been described in detail elsewhere (Ågmo et al. 1991). Briefly, an optical lickometer was mounted in a standard operant cage. Shocks (square pulses of 5 ms duration with a frequency of 100 Hz) were generated by a Grass S48 stimulator connected to a Grass constant current unit adjusted to 0.25 mA. At the test session, shock was applied between the drinking spout and the grid flood for 5 s after every 20 shock free licks. The latency to lick, the total number of licks and the number of licks during shock were registered by a BRS/LVE electromechanical equipment. The lickometer was located in a sound attenuating cage in a room adjacent to the control equipment.

After 24 h of water deprivation, the rats were allowed to drink in the lickometer apparatus for 5 min in the absence of shock. They were then returned to the animal quarters and allowed to drink for another 20 min. Any subject that made fewer than 200 licks in the lickometer was eliminated. This was to ensure that only rats that actually licked were included in the test. A rat that did not lick, or made fewer than 20 licks, would receive no shock, and there would be no conflict. The following day, at the same hour, the 5-min test was made. Here, the drugs were administered before licking and shock was applied as described above. It has previously been shown that this version of the Vogel procedure (Vogel et al. 1971) is not sensitive to variations in motivation to drink, motor effects of drugs or analgesia. Administration of analgesics or increasing the water deprivation from 24 to 48 h do not modify licking at the test. Moreover, benzodiazepines in doses that impair motor execution are ineffective at a test without shock, but increase licking in the presence of shock (Ågmo et al. 1991).

The elevated plus-maze consisted of two opposing open arms (50×10 cm^2) and two enclosed arms ($50 \times 10 \times 40$ cm^3 high), united by a central platform (10×10 cm^2). The apparatus was elevated to a height of 50 cm above floor level. At the beginning of the 5-min test, the rat was placed on the central platform with the

head facing an open arm. Behavior was recorded from above on videotape, and the number of arm entries and the duration of the visits on each arm were anlayzed later. A rat was considered to be on the central platform whenever two paws were posed there, and on an arm when all four paws were on it.

The test for motor impairment was performed with a rotarod. The apparatus consisted of a cylinder (diameter 16 cm) that rotated at 11 rpm. Before the test, the subjects had been trained to walk on the cylinder as described elsewhere (Ågmo et al. 1987). Immediately after a rat had fallen down from the cylinder it was replaced on it, and the number of falls during a 3-min test constituted the measure of motor deficiency. Provisions were taken to avoid any harm to the animals during training and test.

Drugs

Chlordiazepoxide HCl (Roche de México), morphine HCl (Ministry of Health, Mexico), naloxone HCl (Rhône-Poulenc Farma, Mexico) and sodium pentobarbital (Smith Kline and French de México) were dissolved in physiological saline and injected intraperitoneally (IP) in a volume of 1 ml/kg. Diazepam (Roche de México) was suspended in physiological saline to which 2 drops of Tween 80 had been added. The drug was injected IP in a volume of 2 ml/kg. Control injection consisted always of an equivalent volume of saline or saline + Tween 80.

The interval between drug injection and behavioral observation was 15 min for naloxone and pentabarbital, 30 min for chlordiazepoxide and diazpam, and 1 h for morphine.

Design

In the anticonflict and plus-maze experiments, a parallel groups design was used. All doses of a given drug or combination of drugs plus control were run at the same session. Ten subjects received each treatemnt in the Vogel procedure, and there were eight animals per treatment in the plus-maze procedure. Since it was not always practically possible to run all subjects at one session, smaller groups (three to five rats) were sometimes used. This was then repeated until a total of ten rats had received each treatment. On a few occasions, some subjects in the Vogel procedure had to be deleted because of power failure. They were not replaced because of technical reasons. Therefore, there are fewer than ten animals in some groups. This is always indicated in the Results section. No subject was used in more than one experiment.

A repeated measures design was used for the motor execution experiments in such a way that each subject received saline + saline and drug + two doses of naloxone. The order of treatment was randomized for each subject, and the interval between sessions was 48 h.

Statistical analysis

Parameters of licking or plus-maze behavior were anlayzed by one-factor ANOVAs for each drug or combination of drugs. The Hartley F_{max} test for homogeneity of error variances was always performed. In the case of nonhomogeneous error variances, data were analyzed with the Kruskal-Wallis nonparametric ANOVA. A posteriori comparisons were made with Turkey's HSD procedure or the Mann-Whitney *U*-test.

Data from the motor execution experiments were evaluated with Friedman's ANOVA followed by the Wilcoxon matched-pairs signed-ranks test. The use of parametric tests was excluded because of a drastically skewed distribution of the data. Most animals made zero falls after control treatments. All probabilities given are two-tailed.

Results

Chlordiazepoxide, diazepam and pentobarbital increased the total number of licks and the licks during shock without modifying lick latency (Table 1). The 2.5 mg/kg dose of chlordiazepoxide had a partial anticonflict effect, since only the total number of licks was increased. The minimum effective dose of diazepam

Table 1 Parameters of licking after treatment with chlordiazepoxide (*CDO*), diazepam, pentobarbital (*PENTO*), morphine, and naloxone. Doses in mg/kg. Data are means ± SE

Treatment	Number of licks during shock	Total number of licks	Lick latency (s)	*n*
Vehicle	14.1 ± 2.72	143.5 ± 25.91	38.0 ± 29.40	10
CDO 2.5	24.6 ± 6.79	284.4 ± 50.01*	46.7 ± 22.34	10
CDO 5	55.3 ± 5.69***	709.3 ± 13.96***	5.0 ± 1.12	9
Vehicle	19.1 ± 5.16	258.6 ± 71.30	6.2 ± 4.17	10
Diazepam 0.5	25.4 ± 3.63	328.3 ± 44.48	5.0 ± 1.17	10
Diazepam 1	69.8 ± 16.85*	586.9 ± 50.93***	9.4 ± 2.17	10
Diazepam 2	141.0 ± 55.60*	596.9 ± 72.20***	14.2 ± 8.58	10
Vehicle	15.9 ± 2.11	204.1 ± 33.47	14.5 ± 5.89	10
PENTO 1.25	16.0 ± 7.05	185.8 ± 66.62	75.3 ± 38.55	10
PENTO 2.5	29.2 ± 3.67**	367.5 ± 41.27**	12.2 ± 3.53	10
PENTO 5	85.9 ± 14.95***	659.9 ± 44.89***	30.8 ± 16.29	10
PENTO 10	213.9 ± 57.93**	720.0 ± 74.90***	14.1 ± 3.54	10
Vehicle	18.2 ± 5.63	219.1 ± 36.06	26.2 ± 19.69	10
Morphine 5	23.2 ± 6.31	386.2 ± 98.86	28.0 ± 18.59	10
Morphine 10	12.9 ± 5.87	173.2 ± 38.01	18.0 ± 15.31	10
Vehicle	17.4 ± 4.40	204.3 ± 53.90	18.8 ± 5.79	11
Naloxone 2.5	21.3 ± 4.98	229.6 ± 48.82	23.5 ± 12.8	10
Naloxone 5	20.0 ± 6.50	193.2 ± 61.48	40.5 ± 32.53	9
Naloxone 10	14.7 ± 4.47	200.1 ± 63.04	42.4 ± 29.90	10
Naloxone 20	22.6 ± 7.03	218.4 ± 59.84	38.6 ± 27.6	10

Different from vehicle, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

was 1 mg/kg, and of pentobarbital 2.5 mg/kg. Morphine, in doses of 5 and 10 mg/kg, and naloxone, in doses between 2.5 and 20 mg/kg, were ineffective.

When morphine, 5 mg/kg, was combined with a marginally effective dose of chlordiazepoxide, licking was reduced. No effect was found on lick latency. A subeffective dose of diazepam, 0.5 mg/kg, was then combined with morphine, 5 and 10 mg/kg. The former dose was ineffective, while the latter reduced licking, again without affective lick latency. Data are summarized in Table 2.

The results of the interactions between naloxone and the anticonflict drugs are shown in Table 3. The partially effective dose of chlordiazepoxide, 2.5 mg/kg, remained partially effective when it was combined with naloxone, 2.5 mg/kg. Larger doses of naloxone blocked the effect. When chlordiazepoxide was administered in a dose of 5 mg/kg, only the largest dose of naloxone, 10 mg/kg, blocked the anticonflict action. The effects of diazepam, 1 mg/kg, were partially blocked by naloxone, 2.5 mg/kg. Whereas the number of licks during shock was increased, no effect was found on the total number of licks. Higher doses of naloxone completely blocked the effect of diazepam. A diazepam dose of 2 mg/kg was not blocked by naloxone, 2.5 mg/kg, partially blocked by 5 mg/kg, and no difference was obtained between control and diazepam, 2 mg/kg + naloxone, 10 mg/kg.

The anticonflict effect of pentobarbital, 2.5 mg/kg, was blocked by naloxone, 10 mg/kg, but not by lower doses of the opiate antagonist. Because of the large dose of naloxone required to inhibit the effects of pentobarbital, 2.5 mg/kg, it was decided to combine pentobarbital, 5 mg/kg, with naloxone in doses from 5 to

Table 2 Parameters of licking after treatment with morphine in combination with subeffective doses of benzodiazepines. Doses in mg/kg. Data are means ± SE

Treatment	Number of licks during shock	Total number of licks	Lick latency (s)	*n*
Vehicle + vehicle	26.0 ± 2.36	331.1 ± 33.10	12.9 ± 2.99	10
CDO 2.5 + morphine 5	17.0 ± 1.63**	80.1 ± 6.08***	11.9 ± 5.02	10
Vehicle + vehicle	24.5 ± 5.24	313.0 ± 72.63	39.6 ± 18.50	10
Diazepam 0.5 + morphine 5	17.3 ± 2.34	341.9 ± 49.06	13.3 ± 8.51	9
Diazepam 0.5 + morphine 10	15.0 ± 2.94	55.1 ± 5.47***	35.3 ± 22.82	10

Different from vehicle + vehicle, $^{**}P < 0.01$; $^{***}P < 0.001$

Table 3 Parameters of licking in animals treated with different doses of chlordiazepoxide (*CDO*), diazepam, or pentobarbital (*PENTO*) in combination with several doses of naloxone (*NAL*). Doses in mg/kg. Data are means ± SE

Treatment	Number of licks during shock	Total number of licks	Lick latency (s)	*n*
Vehicle + vehicle	16.7 ± 5.02	209.8 ± 48.68	5.4 ± 2.15	8
CDO 2.5 + NAL 2.5	38.7 ± 6.83*	355.0 ± 57.43	28.0 ± 24.47	7
CDO 2.5 + NAL 5	16.4 ± 3.28	195.2 ± 41.80	37.7 ± 19.50	8
CDO 2.5 + NAL 10	17.8 ± 6.29	203.5 ± 55.43	24.9 ± 28.31	8
Vehicle + vehicle	13.5 ± 3.42	206.3 ± 51.69	6.1 ± 1.10	10
CDO 5 + NAL 2.5	77.8 ± 14.0***	433.1 ± 71.19**	42.1 ± 22.74	10
CDO 5 + NAL 5	79.4 ± 19.11**	462.0 ± 86.76**	33.8 ± 29.58	10
CDO 5 + NAL 10	8.2 ± 2.50	143.3 ± 52.31	55.5 ± 30.67	10
Vehicle + vehicle	18.0 ± 3.89	207.9 ± 50.06	32.8 ± 29.71	10
Diazepam 1 + NAL 2.5	43.1 ± 7.16**	338.2 ± 65.21	0.5 ± 0.08	10
Diazepam 1 + NAL 5	18.4 ± 3.06	272.2 ± 48.41	7.3 ± 3.46	10
Diazepam 1 + NAL 10	15.9 ± 4.36	258.3 ± 55.27	12.6 ± 8.83	10
Vehicle + vehicle	13.5 ± 4.08	248.6 ± 52.62	77.6 ± 37.99	10
Diazepam 2 + NAL 2.5	67.3 ± 19.30**	425.9 ± 75.71**	27.2 ± 24.43	10
Diazepam 2 + NAL 5	36.2 ± 4.30**	342.9 ± 41.55	6.8 ± 1.04	10
Diazepam 2 + NAL 10	9.1 ± 5.18	200.8 ± 53.18	134.8 ± 46.15	10
Vehicle + vehicle	18.0 ± 4.32	216.9 ± 46.80	8.6 ± 4.11	10
PENTO 2.5 + NAL 2.5	46.9 ± 6.38***	529.2 ± 53.49***	19.6 ± 14.29	10
PENTO 2.5 + NAL 5	43.5 ± 4.21***	503.2 ± 50.59***	29.3 ± 17.69	10
PENTO 2.5 + NAL 10	17.8 ± 3.15	177.7 ± 36.78	27.5 ± 8.59	10
Vehicle + vehicle	20.1 ± 3.36	235.5 ± 39.12	7.8 ± 4.20	10
PENTO 5 + NAL 5	78.6 ± 4.93***	522.4 ± 44.87***	9.4 ± 7.32	10
PENTO 5 + NAL 10	29.8 ± 4.00	466.6 ± 42.14**	12.1 ± 4.89	10
PENTO 5 + NAL 20	32.0 ± 6.32	323.9 ± 55.57	1.6 ± 0.66	10

Different from vehicle + vehicle, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

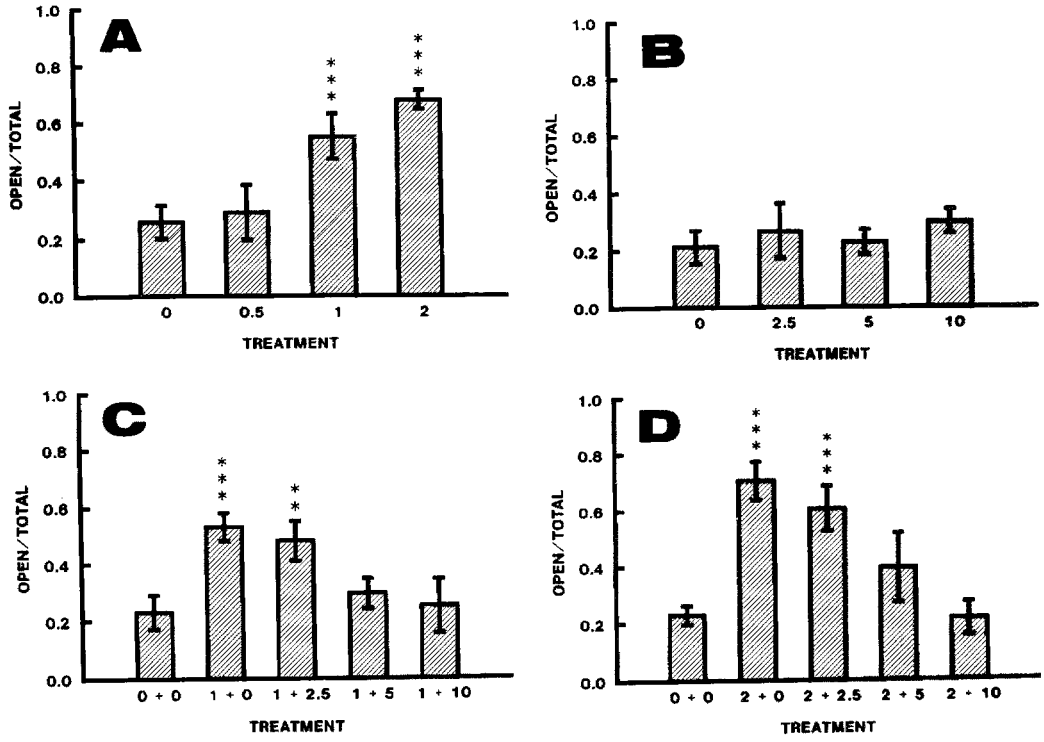

Fig. 1 A–D Proportion of entries in the open arms on the elevated plus-maze in rats treated with different doses of diazepam (**A**), naloxone (**B**), or diazepam, 1 mg/kg (**C**), or 2 mg/kg (**D**) together with several doses of naloxone. Data are mean ± SEM. Doses in mg/kg. $n = 8$ for each treatment. Different from vehicle, $^{**}P < 0.01$; $^{***}P < 0.001$

20 mg/kg instead of the previously used dose range. It turned out, indeed, that the dose of 20 mg/kg was required in order to obtain complete inhibition of the anticonflict effect of pentobarbital. Only a partial inhibition was found after naloxone, 10 mg/kg.

Diazepam, 1 and 2 mg/kg, increased the proportion of entries in the open arms (Fig. 1A) as well as the proportion of time spent in these arms (data not shown). There were no effects on the total number of entries (data not shown). Naloxone, in doses from 2.5 to 10 mg/kg, did not affect any parameter of plus-maze behavior (Fig. 1B). When diazepam, 1 or 2 mg/kg, was combined with naloxone, it was found that the antagonist blocked the effect at doses of 5 and 10 mg/kg (Fig. 1C and D).

In the experiments on motor execution, the minimal effective doses of the benzodiazepines and pentobarbital, previously determined in this laboratory (Ågmo et al. 1991) were used. Since these doses were larger than the ones used in the conflict experiments, the naloxone doses were correspondingly increased. However, naloxone 10 and 40 mg/kg did not reduce the motor incoordination produced by chlordiazepoxide, 12.5 mg/kg, diazepam, 4 mg/kg, or pentobarbital, 10 mg/kg. Data are shown in Fig. 2.

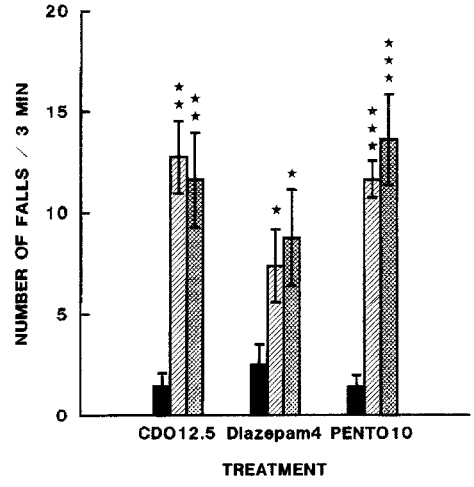

Fig. 2 Motor deficiency produced by chlordiazepoxide (*CDO*), diazepam and pentobarbital (*PENTO*) in combination with naloxone. The effects of the drugs when administered alone have been reported elesewhere (Ågmo et al. 1991). Data are mean ± SEM. Doses in mg/kg. $n = 10$ for each drug. *Black bars* vehicle + vehicle; *striped bars* drug + naloxone, 10 mg/kg; *hatched bars* drug + naloxone 40 mg/kg. Different from vehicle + vehicle, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

Discussion

Present data show that naloxone inhibits the antianxiety activity of chlordiazepoxide, diazepam and pentobarbital in a dose-dependent way. The larger the dose of the agonist, the larger the dose of naloxone required to block the effects. This dose dependency could perhaps explain the contradictory findings reported (see Introduction). An analysis of previously published studies where naloxone was used to block anticonflict effects was therefore made. As can be seen in Table 4, differences in dose cannot explain differences in results. Using the same procedure (open field food intake), Soubrié et al. (1980) blocked the effects of diazepam, 2 mg/kg, with naloxone, 1 mg/kg, whereas

Table 4 Comparison of the results of several studies on anticonflict effects where benzodiazepines have been combined with naloxone

Procedure	Reference	Benzodiazepine and dose	Naloxone dose	Interval drug-test agonist/nal (min)	Effect
Modified Geller-Seifter					
	Billingsley and Kubena 1978	CDO 20 PO	20–100 IP	60/15	–
	Koob et al. 1980	CDO 10 IP	5–10 SC	60/5	–[a]
	Soubrié et al. 1980	Diazepam 2 IP	1 SC	30/15	–
	Duka et al. 1981	Diazepam 1 IP	1–10 IP	30/15	–[b]
	Cannizaro et al. 1987	CDO 5 IP	1 SC	45/30	0
	Tripp and McNaughton 1991	CDO 5 IP	3 IP	15/30	0
Modified Vogel test					
	Billingsley and Kubena 1978	CDO 18 PO	60 IP	60/15	–
Open field food intake					
	Soubrié et al. 1980	Diazepam 2 IP	1 SC	30/15	–
	Britton et al. 1981	Diazepam 1.5 IP	5 SC	30/30[C]	0

Doses in mg/kg. *CDO* chlordiazepoxide, *nal* naloxone, *IP* intraperitoneal, *PO* oral, *SC* subcutaneous, *0*, no effect, –, naloxone blocked the effect of the benzodiazepine
[a]Naloxone 1 mg/kg ineffective
[b]Naloxone 1 mg/kg blocked the effects of diazepem; during the first 30 min of the test but not during the following 30 min. Naloxone 10 mg/kg was effective during the entire session
[c]Cocktail

Britton et al. (1981) were unable to block the effects of diazepam, 1.5 mg/kg, with naloxone, 5 mg/kg. An examination of Table 4 shows that the only reliable difference between positive and negative studies is the interval between naloxone injection and test. The three studies where a long interval (30 min) was used are the only negative ones. Perhaps that naloxone's short half-life (Misra et al. 1976) and its rapid elimination from the brain (Tepperman et al. 1983) can account for the lack of effect. It should be noted that inhibitory effects of naloxone, except the above-mentioned studies, have been found in several procedures supposed to evaluate anxiolytic actions.

Results from the plus-maze experiment show that naloxone is able to block the actions of benzodiazepines in procedures where no experimenter-controlled aversive stimulation is used to suppress behavior. This coincides with other studies using open field food intake as a measure of anxiolytic activity (Soubrié et al. 1980; Britton et al. 1981), and suggests that naloxone antagonism of anxiolysis is a reliable phenomenon. It must be noted, however, that exposure to the plus-maze or to an open field are stressful events, because plasma corticosteroid concentration is much increased (Pellow et al. 1985; Maccari et al. 1991), in fact as much as it is by electric shock (Friedman et al. 1967; Maier et al. 1986).

It appears that the inhibitory action of naloxone is specific to anticonflict effects. The motor impairment produced by benzodiazepines or pentobarbital was not blocked by the opiate antagonist. This latter observation is in agreement with a previous report, where it was found that the muscle-relaxant effect of chlordiazepoxide was not blocked by naloxone (File 1982). The mechanisms behind the effect of naloxone are not clear, but some speculations can be made.

It is unlikely that naloxone acts as an antagonist at the benzodiazepine/GABA/barbiturate/steroid receptor. The binding of diazepam to rat brain synaptosomal membranes is not reduced by opiate antagonists (Möhler and Okada 1977) and the in vivo binding of benzodiazepines is not modified by naloxone (Miller et al. 1987). There is no evidence that naloxone binds to the $GABA_A$ receptor at relevant concentrations (Goldinger et al. 1981), although some antagonism may be found at high micromolar concentrations (Dingledine et al. 1978). It has also been reported that both morphine and naloxone reverse the inhibitory effect of GABA on TBPS binding, an action typical of $GABA_A$ receptor blockers (Jacquet et al. 1987). However, behavioral studies have shown that naloxone, if anything, potentiates the effects of GABA agonists (Ågmo and Tarasco 1985). With regard to the barbiturate binding site, it appears that naloxone is inactive (Olsen and Leeb-Lundberg 1981). At present, it is not known whether naloxone binds to the steroid binding site, but any such action would be of slight or no importance in the present studies. It is interesting to note that the anticonflict effect of benzodiazepines are not blocked by bicuculline or picrotoxin in our version of the Vogel procedure (Ågmo et al. 1991) but the motor impairment produced by these drugs is readily blocked by picrotoxin (Ågmo and Fernandez 1991). This makes it unlikely that the inhibitory effects of naloxone observed here can be attributed to GABA antagonism.

A considerable number of studies have shown that opioids inhibit GABAergic neurons in several brain areas (Nicoll et al. 1980; Cohen et al. 1992; Johnson and North 1992). If, as is widely believed, GABAergic activity is important for the anticonflict actions of benzodiazepines and barbiturates, then morphine would be expected to reduce that activity and naloxone to enhance it. The results from the experiments where morphine was combined with chlordiazepoxide and diazepam seem to support this hypothesis, since licking was reduced by this combination of drugs. However,

the animals licked less than after vehicle, despite the fact that the doses of the benzodiazepines were marginally effective or subeffective. This does not seem to be an inhibition of anticonflict activity. Reduced licking is rather considered to be an indication of proconflict activity (Shephard 1986). However, we have previously reported that the reduced licking produced by several drugs is also observed when the test is made without shock (Ågmo et al. 1991). Reduced licking is therefore not necessarily an indication of proconflict activity. In view of this, we administered chlordiazepoxide, 2.5 mg/kg, + morphine, 5 mg/kg, to a group of animals tested in the absence of shock. As expected, licking was reduced [vehicle + vehicle 1388.4 ± 67.88; chlordiazepoxide + morphine 504.2 ± 107.06, $t(18) = 7.12$, $P < 0.001$]. It appears, then, that some unspecific effect, unrelated to conflict behavior, of this combination of drugs reduces licking. One such unspecific effect may be sedation. This, together with the capacity of naloxone to antagonize the anticonflict effects of chlordiazepoxide, diazepam and pentobarbital, argues against any direct interaction between opiodis and GABAergic neurons in the way described above, in brain regions important for anxiolytic actions.

The doses of naloxone required to inhibit the anticonflict effects are very large. For example, the reinforcing effects of a very large dose of morphine, 10 mg/kg, as evaluated with the conditioned place preference procedure, are completely blocked by naloxone, 1 mg/kg (Ågmo et al. 1992). The cataleptic effects of morphine (20 mg/kg) are also blocked by low doses (0.01 mg/kg) of naloxone (Brown et al. 1983). This would suggest that either naloxone acts nonspecifically at a nonopioid receptor or at an opioid receptor for which it has low affinity, or that a drastic inhibition of opioid systems is necessary.

The first possibility cannot be excluded, but it has been reported that naloxone does not bind significantly to the 5-HT_{1A} (Martin et al. 1991) or the dopamine (Carlsson and Seeger 1982) receptor, receptors that may be important for conflict behavior (see Taylor et al. 1982; Lerner et al. 1986; Gardner 1988).

The second possibility seems more likely. Naloxone has high affinity for the mu receptors and comparably low affinity for kappa receptors (Goldstein 1987). It is possible that the antagonism of anticonflict effects is mediated by the kappa receptor. There is no direct evidence for this hypothesis, but kappa agonists and benzodiazepines share the capacity to inhibit dopaminergic activity (Reinhard et al. 1982; Manzanares et al. 1991; Donzanti et al. 1992; Gruen et al. 1992), something that may be important for anticonflict actions. Furthermore, pentobarbital has several autonomic effects similar to those obtained with kappa agonists, and these actions of pentobarbital are blocked by naloxone (Gilbert and Martin 1977).

As to the third possibility, it could be argued that the release of endogenous opioids associated with a variety of stressors, including electric shock, immobilization, etc. (see Olson et al. 1993 for review; Larsen and Mau 1994), may be important for anticonflict effects. Indeed, it has been reported that CSF concentrations of β-endorphin are associated with anxiety ratings in normal subjects but not in subjects suffering from panic disorder (Brady et al. 1991). The authors suggested that endogenous opioid peptides play a role in the control of anxiety, and that this control is absent in panic disorder. Again, there is no direct evidence for this hypothesis. Nevertheless, the effects of stress on morphine analgesia are dependent on benzodiazepine receptors (Tokuyama et al. 1989). It has also been reported that the release of β-endorphin from the anterior pituitary and neurointermediate lobe in chronically stressed rats is enhanced by alprazolam (Forman et al. 1991). Perhaps large doses of naloxone are required to block relevant consequences of stress-induced opioid release. This, however, is entirely speculative. Further work is needed before a firm hypothesis as to the mechanisms of naloxone's antagonism of anticonflict effects can be proposed.

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