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Selective interaction of homophtalazine derivatives with morphine

Márton I.K. Fekete [a,*], Katalin Horváth [b], Rita Kedves [b], Ildikó Máté [b], József I. Székely [b], Eszter Szentkuti [b]

[a] *EGIS Pharmaceuticals Ltd., Budapest, Hungary*
[b] *Institute for Drug Research, Budapest, Hungary*

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Abstract

Homophtalazines show specific binding sites in the nigrostriatal system and to find their target of action the interactions between these derivatives, nerisopam and girisopam, and chlorpromazine, chlordiazepoxide and morphine were assessed. The compounds did not influence the chlorpromazine induced decrease in motility and catalepsy, nor did they alter the antiaggressive and anticonvulsive action of chlordiazepoxide. However, nerisopam and girisopam augmented the agonist potency of morphine to induce catalepsy or analgesia; they also altered the opioid antagonist potency of naloxone. The naloxone-induced decrease in sucrose consumption in drinking water was augmented by nerisopam and girisopam. It is suggested that a possible target of action of homophtalazines is the opioid signal transduction. © 1997 Elsevier Science B.V.

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1. Introduction

Homophtalazines (also called 2,3-benzodiazepines) are compounds with a characteristic pharmacological profile. The parent compound, tofisopam, is used in human therapy as a non-sedative anxiolytic drug (for references see Pellow and File, 1986). The animal pharmacology of the most active derivatives shows them to have anxiolytic, antiaggressive properties, with relatively moderate sedative and no anticonvulsant and muscle relaxant activities (Andrási et al., 1987; Horváth et al., 1989, 1992a). The mechanism of action of these compounds is still unknown, although actions via the GABA (γ-aminobutyric acid)–benzodiazepine receptor complex as well as effects on dopaminergic signal transduction have been suggested (Saano and Urtti, 1982; Chopin et al., 1985). However, quite recently a binding site for homophtalazines has been demonstrated in the nigrostriatal system of rats, mice, guinea pigs and cats (Salamon et al., 1992; Horváth et al., 1993; Horváth et al., 1994). The finding of specific homophtalazine binding sites in the striatum has stimulated the research to identify the target of homophtalazines' action by studying the interaction profile of these compounds. Nerisopam and girisopam, two highly active derivatives, were used and their interactions with the 1,4 benzodiazepine chlordiazepoxide, the dopamine antagonist chlorpromazine and the opioid receptor agonist morphine were measured in different pharmacological test procedures. Here, we report on the selective interaction of homophtalazines with morphine and its antagonist, naloxone, suggesting a characteristic involvement of opioid signal transduction in their mechanism of action.

2. Materials and Methods

2.1. Husbandry

The animals were purchased from LATI (Breeding Farm of Laboratory Animals, Gödöllő, Hungary) and were kept for 3–6 days after arrival in their housing room before use in acute experiments. The room temperature was kept at 22 ± 1°C with 40–50% relative humidity and regular lighting (lights on from 7.00 a.m. until 7.00 p.m.). Free access to standard semisynthetic food pellets

* Corresponding author. Present address: Institute for Experimental Medicine, Hungarian Academy of Sciences, P.O. Box 67, H-1450 Budapest 8, Hungary. Tel.: (36-1) 210-0819, ext. 268; Fax: (36-1) 210-0813; e-mail: fekete@koki.hu

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(purchased from LATI) and tap water was allowed. The experiments were carried out between 9 and 12 a.m. The experimental procedures were carried out with the permission of the local Ethical Committee.

2.2. Spontaneous motility in mice

Male CFLP (Carworth Farm Lane Petter) mice of 18–22 g body weight were used. After a fasting period of 18 h the animals were treated with the test compounds and the motility counts of 4–8 groups of 3 mice were measured at each dose used. Motility was determined in every 10 min for 2 h. Horizontal movements were registered by changes in the capacitance of the insulated metal floor of boxes used for the motility measurements.

2.3. Measurement of catalepsy in rats

The method of Costall and Naylor (1973) was used with a five-channel automatic apparatus, which enabled us to measure the time spent in immobile posture. Male Sprague-Dawley rats of 250–300 g body weight were placed with their forelimbs on a horizontal rod (diameter 1 cm), which was 10 cm above the test surface. For 2–4 h the time spent in this posture was measured every 30 min. The catalepsy was scored as shown in Table 1.

Naloxone, morphine and chlorpromazine were dissolved in saline, whereas the test compounds were administered in a Tween 80 (2%, v/v) suspension. Naloxone was administered s.c. 15 min before the simultaneous i.p. injection of morphine or chlorpromazine and one of the test compounds; 10 animals were used per groups.

2.4. Fighting mice test

The method of Tedeschi et al. (1959) was used with male CFLP mice of 18–22 g body weight. Pairs of mice were selected which showed at least 3 fighting episodes after application of footshocks (2 Hz, 3 mA) through the grid floor during the 3 min control examination. The animals were treated with test compounds 1 h before testing and the number of fighting episodes were counted again. Homophtalazines were administered i.p. simultaneously with chlordiazepoxide given orally; 10 pairs of mice were examined in each group.

Table 1
Catalepsy scores

Time (min) spent in cataleptic posture	Score
0	0
0.25–2.5	1
2.60–5.0	2
5.10–10.0	3
10.1–20.0	4
> 20.0	5

2.5. Anticonvulsant activity

CFLP male mice of 22–28 g body weight were used. Convulsions were induced either by maximal electroshock (15 mA current of 150 Hz and 0.2 ms pulse width delivered by ear clips for 0.2 s; Swinyard et al., 1952), or by pentylenetetrazol (43 mg/kg, i.v.), as described by Goodman et al. (1953). Both electric shock and pentylenetetrazol elicited generalized tonic extension. After an 18 to 24 h fasting period three increasing oral doses of chlordiazepoxide were given 60 min before and homophtalazines were injected i.p. 30 min before maximal electroshock or pentylenetetrazol injection. Animals were considered to be protected when they showed no tonic extension of hindlimbs in the case of maximal electroshock, or 24 h survival in the case of pentylenetetrazol. At least 10 animals were used per dose.

2.6. Analgesia

2.6.1. Treatment schedules

The opioid receptor antagonist naloxone was injected s.c. 30 min before morphine (s.c.). Girisopam or nerisopam was injected i.p. 15 min before morphine. Girisopam and nerisopam were tested in vehicle treated animals or in combination with morphine or naloxone or both. In each experiment three injections were administered and one or two drugs were eventually replaced by saline.

2.6.2. Tail flick

Male rats (160–180 g body weight) of CFY/Harlem strain were used. The method of D'Amour and Smith (1941) was used. Animals were gently wrapped in soft tissue paper and the tail was positioned over a photocell. The light from a projection bulb was focused on the tail at a point about 30–40 mm from the tip. Heat induced removal of the tail exposed the photocell to the light and stopped the clock measuring the reaction time to the nearest 0.1 s. The intensity of the light beam was set so that the control latency time varied between 3 and 5.0 s. The latency time was measured once before treatment and 15, 30, 45 and 60 min afterwards. The cut off time was 15 s.

For data analysis first the 'mean possible analgesia' (% MPA) was calculated according to the formula

$$\%\mathrm{MPA} = \frac{\mathrm{test}_{\mathrm{latency}} - \mathrm{control}_{\mathrm{latency}}}{\mathrm{cutoff} - \mathrm{control}_{\mathrm{latency}}}$$

The doses of drug under study were plotted against the maximal % MPA (determined at any posttreatment reading) and the data were analyzed by least-squares regression analysis. The ED_{50} values ($\pm$95% confidence intervals) and slopes were calculated according to Bolton (1990).

2.7. Measurement of drinking of sucrose solution

Male rats (Wistar, Charles River) of 240–260 g body weight were used. Following a week of adaptation, the consumption of water and 10% (w/v) sucrose (commercial sugar) was measured in individual rats after 18 h of water deprivation. Each rat had free access to two identical bottles, one filled with tap water and the other containing sucrose solution. Consumption was measured by weighing the bottles before and 10, 30 min after the beginning of the session. The fluid consumption was measured for four consecutive days. During this period, the drinking habits of the rats stabilized; they consumed 4–5 times more sucrose than tap water. On the fifth day 40 min before testing girisopam or nerisopam was given i.p. and naloxone was injected s.c. 30 min before the measurement. The fluid consumption was measured thereafter as described above.

2.8. Drugs

Morphine sulphate was obtained from Alkaloida (Tiszavasvári, Hungary), chlordiazepoxide from G. Richter (Budapest, Hungary) and chlorpromazine from EGIS Pharmaceuticals (Budapest, Hungary). Pentylenetetrazole and naloxone were purchased from Sigma. Homophtalazines, i.e., girisopam (1-(3-chlorophenyl)-4-methyl-7,8-dimethoxy-5H-2,3-benzodiazepine) and nerisopam (1-(4-aminophenyl)-4-methyl-7,8-dimethoxy-5H-2,3-benzodiazepine) were synthesized as described (Kőrösi and Láng, 1974; Kőrösi et al., 1981). Morphine and naloxone were dissolved in saline and girisopam and nerisopam were suspended in 1% Tween 80. Drug solutions were administered in volumes of 0.1 ml/10 g body weight in mice and 0.2 ml/100 g body weight in rats.

2.9. Data analyses

ED_{50} values with 95% confidence intervals were calculated according to Litchfield and Wilcoxon (1949), using a computer adapted version. The same method was used to compare ED_{50} values. The results of movement tests, catalepsy measurements and sucrose consumption were calculated using ANOVA (analysis of variance) followed by Newman–Keuls or Dunnett's test or Kruskal–Wallis test. The data of the tail flick assay were processed as described in Section 2.6.2. The calculations were performed with the aid of SAS (Statistical Analysis Systems, New York, NY, USA).

Table 2
Effect of chlordiazepoxide and nerisopam or girisopam on maximal electroshock seizures

Treatment	ED_{50} mg/kg p.o.	95% Confidence limit	*n*
Chlordiazepoxide	28.9	24.2–34.4	30
Chlordiazepoxide + girisopam 10 mg/kg	32.4	27.4–38.4	40
Chlordiazepoxide + nerisopam 10 mg/kg	36.7	25.2–53.4	40

Chlordiazepoxide was given in doses of 20–40 mg/kg p.o. 60 min before electric shock, nerisopam and girisopam were administered 30 min after chlordiazepoxide intraperitoneally.

Table 3
Inhibition of pentylenetetrazol convulsion by chlordiazepoxide, effects of nerisopam and girisopam

Treatment	ED_{50} mg/kg p.o.	95% Confidence limit	*n*
Chlordiazepoxide	2.06	1.39–3.06	50
Chlordiazepoxide + girisopam 10 mg/kg	1.36	0.81–2.29	40
Chlordiazepoxide + nerisopam 10 mg/kg	1.48	1.06–2.06	40

Chlordiazepoxide was given in doses of 1.25–5.0 mg/kg p.o. 60 min before pentylenetetrazol, and nerisopam and girisopam were administered 30 min after chlordiazepoxide intraperitoneally. Pentylenetetrazol was given i.v. in a dose of 43 mg/kg.

3. Results

3.1. Interaction with chlordiazepoxide

3.1.1. Anticonvulsant activity

The well established anticonvulsant activity of chlordiazepoxide could be demonstrated both in electroshock and in pentylenetetrazol convulsion tests. Neither girisopam nor nerisopam altered the anticonvulsant activity of this 1,4-benzodiazepine (Tables 2 and 3). The homophtalazine

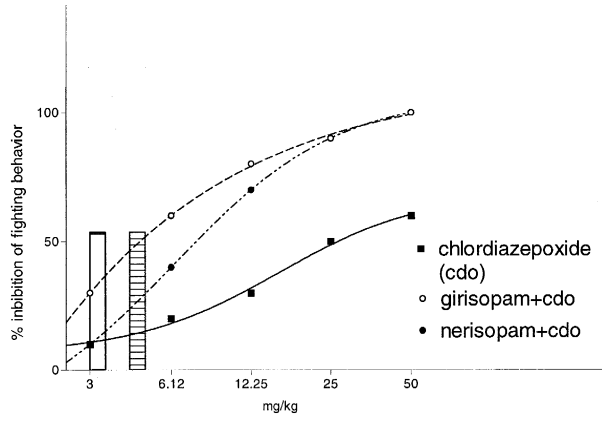

Fig. 1. The effects of nerisopam, girisopam and chlordiazepoxide in the fighting mice test. The interaction of 5 mg/kg i.p. administered nerisopam and 10 mg/kg i.p. girisopam with increasing doses (abscissa) of orally given chlordiazepoxide was examined. Treatments were given 1 h before measurements. Ordinate: percent inhibition of fighting behavior. At each points 10 pairs of mice were examined. Open columns: inhibition of fighting by girisopam (10 mg/kg i.p.), hatched columns: inhibition by nerisopam (5 mg/kg i.p.) given alone.

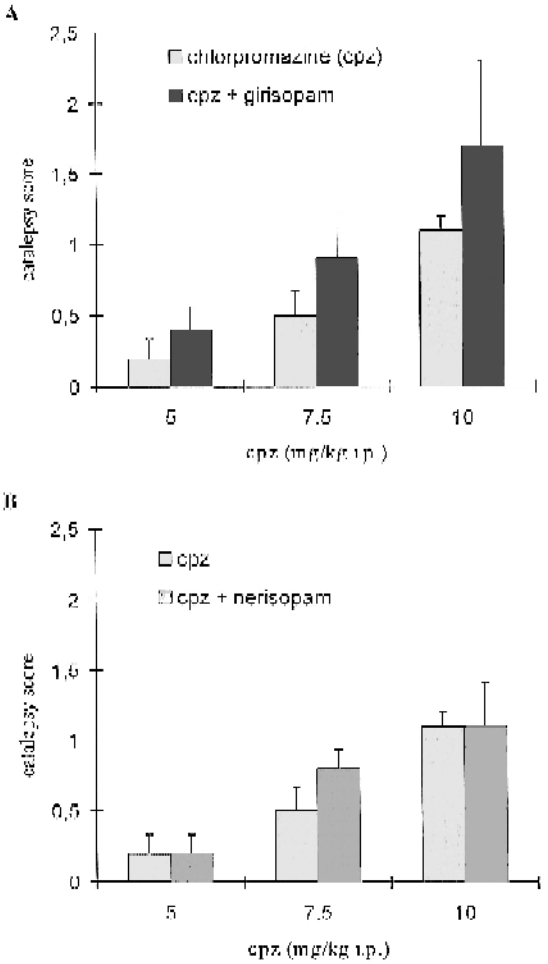

Fig. 2. Chlorpromazine induced catalepsy and the effect of (A) girisopam (50 mg/kg) and (B) nerisopam (10 mg/kg). Rats were treated i.p. 150 min before measurements (time of maximal effect of chlorpromazine). Mean values ± S.E.M., $n = 10$.

derivatives alone in the doses used did not alter the electroshock or pentylenetetrazol seizures.

3.1.2. Fighting mice test

Chlordiazepoxide inhibited fighting behavior in mice, with an oral ED_{50} of 29.2 (13.3–64.7) mg/kg. The calculated ED_{50} values of girisopam and nerisopam were 7.75 (4.79–12.55) and 4.03 (2.52–6.45) mg/kg, respectively. The chlordiazepoxide dose–response curve was shifted to the left by simultaneous administration of 5 mg/kg nerisopam or 10 mg/kg girisopam (Fig. 1). The shifts of the curves were significant ($p < 0.05$, calculated according to Litchfield and Wilcoxon, 1949) and suggest that the effects of homophtalazines and chlordiazepoxide were additive.

3.2. Interaction with chlorpromazine

3.2.1. Motility

Chlorpromazine was administered in doses of 1.5, 6.25 and 25 mg/kg orally. Girisopam (10 mg/kg i.p.) and nerisopam (5 mg/kg i.p.) caused a significant decrease in motility (see inset to Fig. 3). This decrease caused by girisopam and nerisopam in animals treated with the above doses of chlorpromazine was not significantly different from that caused by the homophtalazine derivatives alone (data not shown).

3.2.2. Catalepsy

Chlorpromazine in doses of 5–10 mg/kg i.p. induced dose-related catalepsy in rats, with a maximum at 150 min after treatment. Pretreatment with 10 mg/kg nerisopam or 50 mg/kg girisopam did not modify the cataleptogenic activity of chlorpromazine (Fig. 2). The slight increase in

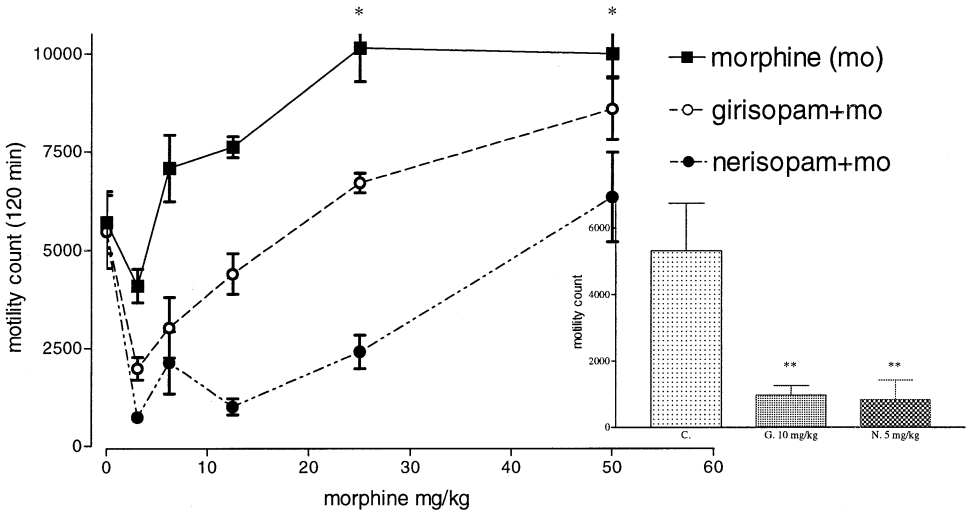

Fig. 3. Morphine-induced hypermotility in mice and the effects of girisopam and nerisopam. The doses of girisopam and nerisopam i.p. were 10 and 5 mg/kg, respectively. Treatments were given simultaneously at 0 min. Mean values ± S.E.M., 9–15 mice were examined at each points. * The effect of morphine is significant ($F = 38.0$, Newman–Keuls test: $P < 0.05$), the effects of girisopam ($F = 52.5$) and nerisopam ($F = 218.7$) compared to the morphine-treated group are significant at each point of measurement ($P < 0.001$). Inset: the effects of the doses of girisopam and nerisopam used on motility of mice; ** $P < 0.01$, $F = 10.99$ (ANOVA followed by Newman–Keuls test).

the scores of the simultaneously treated groups was not significant by ANOVA followed by the Kruskal–Wallis test.

3.3. Interaction with morphine

3.3.1. Motility

Morphine in doses above 5 mg/kg s.c. increased the spontaneous motility of mice. Nerisopam (5 mg/kg i.p.) and girisopam (10 mg/kg i.p.) inhibited this effect of morphine (Fig. 3). There was a rightward parallel shift of the morphine dose–response curve in both cases. Nerisopam and girisopam inhibited the motility of mice similarly, when administered alone (Fig. 3, inset).

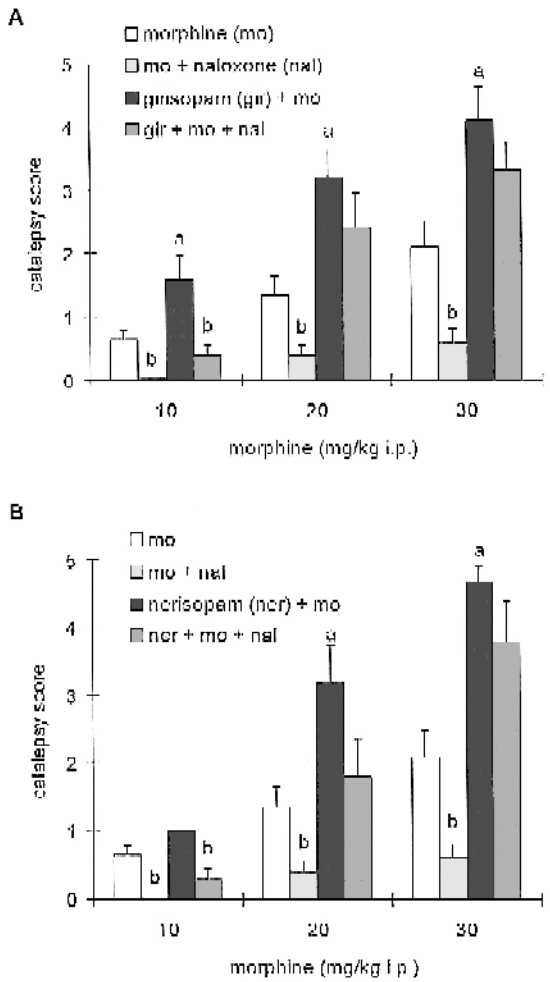

Fig. 4. The effects of nerisopam and girisopam on morphine-induced catalepsy in rats. Increasing doses of morphine (abscissa) as well as (A) girisopam (50 mg/kg) and (B) nerisopam (10 mg/kg) were given i.p. 45 min before measurements (time of maximal catalepsy evoked by morphine). Naloxone was administered in a dose of 10 μg/kg s.c. 15 min before simultaneous injection of morphine and homophtalazines. Mean ± .S.E.M., $n = 10$; (a) the effect of nerisopam or girisopam is significant $P < 0.01$ ($F = 30.4$), (b) the effect of naloxone is significant $P < 0.001$ ($F = 27.8$) compared to the respective morphine treated group (Kruskal–Wallis test following ANOVA).

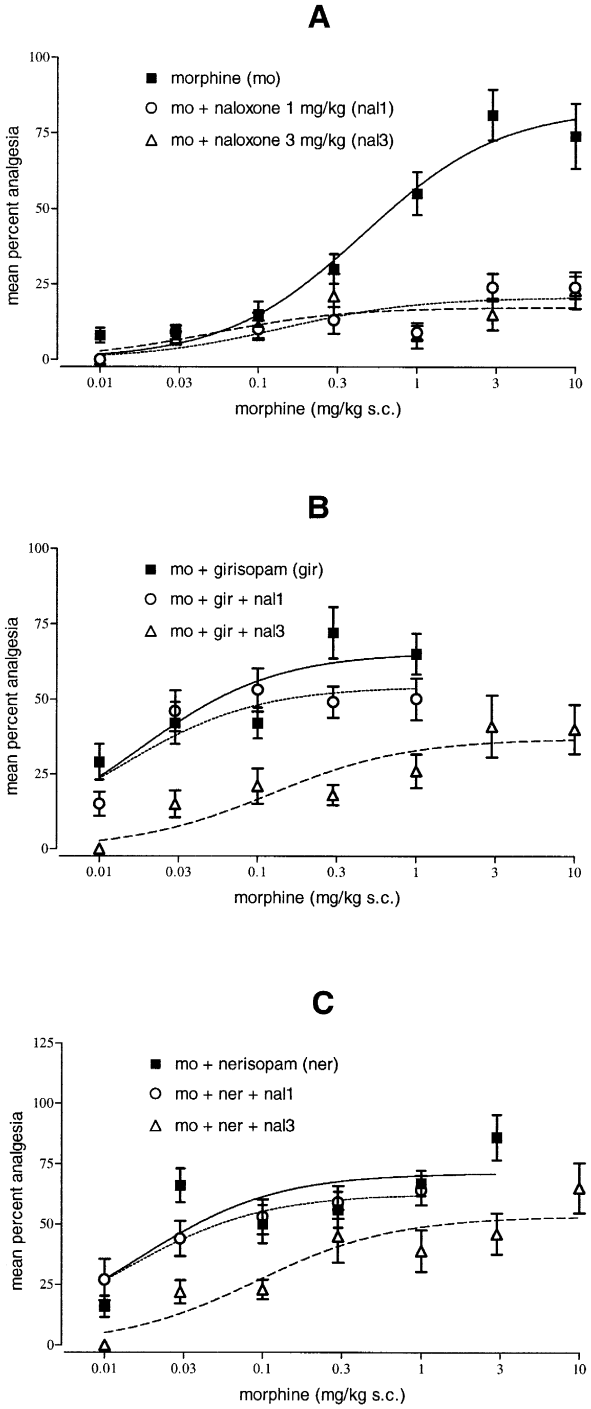

Fig. 5. Analgesic action of morphine in rat tail flick test and its antagonism by naloxone in rats treated with (A) saline, (B) girisopam (10 mg/kg i.p.), (C) nerisopam (10 mg/kg i.p.) 15 min prior to s.c. injection of morphine. Saline or naloxone pretreatments were given 30 min before morphine administration. Measurements were taken 30 min after the injection of morphine. Mean values ± S.E.M, at each points 10 animals were used.

3.3.2. Catalepsy

The catalepsy scores after morphine injection (Fig. 4) were significantly increased by girisopam ($F = 27.6$, $P <$

Table 4
Effects of naloxone, girisopam or nerisopam on analgesic ED_{50} values of morphine

Treatment	ED_{50} (mg/kg)		
	Control	Naloxone 1 mg/kg	Naloxone 3 mg/kg
Morphine + saline	0.87 (0.072–1.491)	> 10	> 10
Morphine + girisopam 10 mg/kg	0.097 (0.038–0.210)	0.33 (0.084–277.2)	> 10
Morphine + nerisopam 10 mg/kg	0.053 (0.043–0.276)	0.095 (0.029–0.244)	2.811 (0.949–29.3)

For other conditions see the legend to Fig. 5. Calculations were done by least-squares linear regression analysis. 95% confidence limits are given in parentheses.

0.001) and nerisopam ($F = 45.2$, $P < 0.001$). The catalepsy scores for nerisopam and girisopam alone were 0.7 ± 0.15 and 0.1 ± 0.10, respectively. Naloxone in a dose of 10 μg/kg s.c. almost totally inhibited the catalepsy evoked by morphine in controls ($F = 14.6$, $P < 0.001$). This antagonism was much weaker in girisopam- and nerisopam-treated animals.

3.3.3. Analgesia

Girisopam or nerisopam alone did not induce analgesia in the rat tail flick test (up to a dose of 80 and 40 mg/kg, respectively). Higher doses were not examined because of induction of motor incoordination.

The analgesic potency of morphine was increased about 10–15 times by 10 mg/kg of girisopam or nerisopam (Table 4). These parallel shifts of the morphine dose–response curve to the left were highly significant ($P < 0.001$) according to the regression analysis. The potency of naloxone to antagonize the analgesic action of morphine was also examined after pretreatment with girisopam or nerisopam. In saline treated rats the analgesic dose–response curve of morphine was shifted to the right by increasing doses of naloxone. A decreased shift was observed in girisopam- or nerisopam-treated rats at the dose of 1 mg/kg of naloxone. However, at the dose of 3 mg/kg of naloxone the inhibitory potency of the antagonist was apparently not changed or even increased (Fig. 5, Table 4).

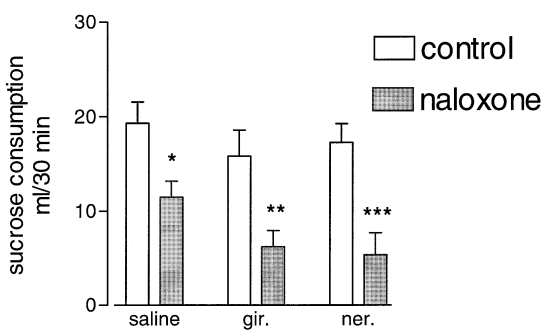

Fig. 6. Effect of naloxone (nal) 0.66 mg/kg s.c., girisopam (gir) 20 mg/kg i.p. and nerisopam (ner) 10 mg/kg i.p. on sucrose consumption by rats. Mean values ± S.E.M. ($n = 9$–12). * $P < 0.05$ compared to the saline treated control; ** $P \sim 0.05$, *** $P < 0.025$ compared to the saline- and naloxone-treated group, $F = 7.12$ (ANOVA followed by Newman–Keuls test).

3.3.4. Sucrose consumption

The naloxone dose dependently inhibited the sucrose consumption in rats (data not shown). The dose of 0.66 mg/kg was selected to measure the interaction with homophtalazines. In saline pretreated rats this dose caused a 40–50% inhibition of sucrose consumption. Nerisopam and girisopam alone induced only a slight and non-significant decrease in sucrose consumption. Both homophtalazine derivatives augmented the inhibition caused by naloxone (Fig. 6). The same doses of naloxone, girisopam and nerisopam caused no significant alteration in the drinking of water (data not shown).

4. Discussion

The detection of specific binding sites for homophtalazines in the nigrostriatal system (Salamon et al., 1992; Horváth et al., 1993, 1994) suggested a specific signal transduction process as their target of action. To test this hypothesis, as a first step interactions with psychotropic compounds were examined. Indeed, by measuring the interactions of homophtalazines we could reveal characteristic changes induced by these compounds in the action of the opioid receptor agonist morphine and its antagonist naloxone.

Girisopam and nerisopam, highly active homophtalazines in behavioral tests in rodents (Andrási et al., 1987; Horváth et al., 1989, 1992a), enhanced morphine-induced catalepsy and analgesia, whereas they inhibited the hypermotility caused by this opioid receptor agonist in mice. The morphine antagonist potency of small doses of naloxone was reduced by girisopam and nerisopam in the catalepsy and analgesia tests.

An increased sensitivity to naloxone could be demonstrated by measuring the effect of naloxone on sucrose consumption. The consumption of water and especially of palatable sucrose solution is sensitive to opioids (Cooper et al., 1985; Koch et al., 1995). The changes in this behavioral response may indicate an action of homophtalazines on opioid signal transduction influencing motivation.

The hypermotility caused by opioid agonists is inhibited by pharmacological manipulation of central dopaminergic/noradrenergic neurotransmission (reserpine, α-methyltyrosine), or by simultaneous administration of a dopamine

antagonist. Therefore this effect of opioids is thought to be mediated via indirect dopaminergic mechanisms (Engel, 1977; Zarrindast and Zarghi, 1992). Homophtalazines inhibited the morphine-induced hypermotility.

Girisopam and, more characteristically, nerisopam, show some similarity to the dopamine antagonist neuroleptics (Andrási et al., 1987; Horváth et al., 1989). They inhibit apomorphine-climbing and the amphetamine-induced hypermotility. Moreover, nerisopam is active in inhibiting conditioned active avoidance responses in rats (Horváth et al., 1989). However, in assays, which reflect dopamine antagonist character (effects on dopamine D_2 receptor ligand binding, dopamine sensitive adenylate cyclase and serum prolactin level) the homophtalazines are inactive (Andrási et al., 1987; Horváth et al., 1989). These data and the presently demonstrated lack of interaction with chlorpromazine seem to exclude the possibility of a direct action of homophtalazines on dopaminergic receptor systems.

The mechanism of catalepsy induced by opioids differs markedly from that of haloperidol-, arecoline- and GABA-induced immobility (Rondeau et al., 1982). Muscarinic (dominantly M_1) receptor antagonists augment, whereas nicotinic receptor antagonists decrease the catalepsy induced by methadone (Ahtee, 1976). In addition, Ezrin-Waters et al. (1976) found anticholinergic agents to inhibit the catalepsy induced by haloperidol, but not that caused by morphine. They raised the possibility of striatal and non-striatal (amygdala) origins of the drug-induced motor disturbance. Similarly, a non-striatal catalepsy-inducing action of morphine was suggested by Costall and Naylor (1974). These authors also suggested the role of serotonin in opioid catalepsy. The cataleptogenic action of opioids, but not the analgesic action, is antagonized by cholera toxin (Massi et al., 1993). Interestingly, dermorphine-induced catalepsy can be antagonized by a 1,4-benzodiazepine antagonist (Paakkari and Feuerstein, 1988). The possible role of dopamine D_1 receptor-mediated processes in the catalepsy induced by morphine was suggested by the finding that chronic treatment with a selective dopamine D_1 receptor antagonist inhibits the extrapyramidal effects of opioid receptor agonists (De Montis et al., 1989). Thus a possible explanation for the effects of homophtalazines is that they alter dopamine D_1 receptor mediated signal transduction downstream of the dopamine-induced changes in second messengers.

Potentiation of opiate effects has been known and described for various chemical group of compounds acting on different receptors of the central nervous system. One of the most widely analyzed neurotransmitter systems influencing morphine analgesia, tolerance and other opiate effects is the GABA system. GABAergic agents per se (e.g., agonists, GABA-transaminase inhibitors, etc.) induce analgesia in various animal models of antinociception, although relatively high doses are needed to achieve this effect (Kendall et al., 1982; Cheng and Brunner, 1985; Sawynok, 1987; Aley and Kulkarni, 1991; Johnston, 1992; Holmes and Fujimoto, 1994). However, the analgesia evoked by GABAergic drugs differs from that induced by opioids because it is naloxone-insensitive and it can be elicited even in morphine-tolerant animals (Sivam and Ho, 1983). Co-administration of GABA receptor agonists or compounds enhancing endogenous GABA neurotransmission (e.g., GABA transaminase inhibitors) and opioid receptor agonists increases the level of opiate analgesia (Sivam and Ho, 1985).

Similarly, benzodiazepines, acting on the benzodiazepine-GABA-chloride ion channel receptor complex, can also modulate opiate effects in the same fashion as GABAergic drugs do. Rattan and Sribanditmonghol (1994) found that the benzodiazepine receptor agonist, midazolam, enhanced morphine-induced lethality, catalepsy and analgesia. Bianchi et al. (1993) reported that peripheral administration of alprazolam, chlordiazepoxide and midazolam enhanced morphine-induced analgesia in rats, using the tail flick method. This effect could be reversed by administration of the benzodiazepine receptor antagonist Ro 15-3505 or the inverse agonist FG 7142 indicating that the modulation of the nociceptive responses involved 1,4-benzodiazepine receptors. The interaction between GABAergic and opioid peptide systems was also demonstrated by Rocha et al. (1993), who found that morphine, met-enkephalin or naloxone influence benzodiazepine receptor binding.

These data indicate that compounds with apparent anxiolytic activity can enhance certain behavioral effects of opioids. In this respect, it is not surprising that 2,3-benzodiazepines, which are active in different animal models of anxiolysis, potentiated morphine-induced catalepsy and antinociception. In earlier studies no interaction with central 1,4-benzodiazepine binding was found, whereas at relatively high (40–100 μM) concentrations girisopam decreases the affinity of [^{3}H]1,4-benzodiazepine receptor ligands for peripheral benzodiazepine receptors (Kenessey et al., 1987) and slightly increases only low-affinity GABA binding (Páldi-Haris et al., 1985), although a structural analog, the parent compound tofisopam, is claimed to modulate 1,4-benzodiazepine receptor affinity in rat brain (Saano and Urtti, 1982). 1,4-Benzodiazepines do not displace ligands from homophtalazine binding sites (Salamon et al., 1992). Accordingly, girisopam and nerisopam showed no anticonvulsant activity (Andrási et al., 1987; Horváth et al., 1989, 1992b) and nor did they modify the anticonvulsant and antiaggressive activity of a 1,4-benzodiazepine, chlordiazepoxide. The rather negligible action of homophtalazines on 1,4-benzodiazepine receptor mediated effects can not explain the relatively strong synergestic effect of girisopam and nerisopam with morphine in the present experiments measuring analgesia and catalepsy.

The results of receptor binding studies in progress show that in vitro treatment of striatal membranes with homophtalazines results in an increase in the number of naloxone

binding sites. Further studies are needed to clarify the biochemical mechanism of this altered binding capacity. We suggest that girisopam and nerisopam modulate opioid signal transduction in certain areas of the brain. This structurally localized action in the striatum and nucleus accumbens (Salamon et al., 1992) may explain changes in emotional status, and consequently an altered motivation, which may explain the effects of homophtalazines in behavioral tests for anxiolytic activity.

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References

Ahtee, L., 1976. Effect of cholinergic drugs on methadone-induced catalepsy and stereotypies in rats treated chronically with methadone. Eur. J. Pharmacol. 39, 203–213.

Aley, K.O., Kulkarni, S.K., 1991. Baclofen analgesia in mice: A GABA B-mediated response. Methods Find. Exp. Clin. Pharmacol. 13, 681–686.

Andrási, F., Horváth, K., Sineger, E., Berzsenyi, P., Borsy, J., Kenessey, Á., Tarr, M., Láng, T., Kőrösi, J., Hámori, T., 1987. Neuropharmacology of a new psychotropic 2,3-benzodiazepine. Arzneimittelforschung 37, 1119–1124.

Bianchi, M., Mantegazza, P., Tammiso, R., Zonta, N., Zambotti, F., 1993. Peripherally administered benzodiazepines increase morphine-induced analgesia in the rat. Arch. Int. Pharmacodyn. Ther. 322, 5–13.

Bolton, S., 1990. Pharmaceutical Statistics. Marcel Decker, New York/Basel.

Cheng, S.C., Brunner, E.A., 1985. Inducing anesthesia with a GABA analog, THIP. Anesthesiology 63, 147–151.

Chopin, P., Stenger, A., Briley, M., 1985. Tofisopam, a 3-4 benzodiazepine, potentiates dopaminergic stimulation. J. Pharm. Pharmacol. 37, 917–919.

Cooper, S.J., Jackson, A., Morgan, R., Carter, R., 1985. Evidence for opiate receptor involvement in the consumption of a high palatability diet in nondeprived rats. Neuropeptides 5, 345–348.

Costall, B., Naylor, R.J., 1973. Neuroleptic and non-neuroleptic catalepsy. Arzneimittelforschung 23, 674–682.

Costall, B., Naylor, R.J., 1974. A role of amygdala in the development of the cataleptic and stereotypic actions of narcotic agonists and antagonists in the rat. Psychopharmacology 35, 203–213.

D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

De Montis, M.G., Devoto, P., Meloni, D., Porcella, A., Saba, P., Tagliamonte, A., 1989. Resistance to extrapyramidal effects of opiates in rats chronically treated with SCH 23390. J. Neurosci. Res. 24, 286–292.

Engel, J., 1977. Neurochemical aspects of euphoria induced dependence-producing drugs. In: Idestrhom, C.-M. (Ed.), Recent Advances in Study of Alcoholism. Excerpta Medica, Amsterdam, pp. 16–22.

Ezrin-Waters, C., Muller, P., Seeman, P., 1976. Catalepsy induced by morphine or haloperidol: Effects of apomorphine and anticholinergic drugs. Can. J. Physiol. Pharmacol. 54, 516–519.

Goodman, L.S., Grewal, M.S., Brown, W.C., Swinyard, E.A., 1953. Comparison of maximal seizures evoked by pentylene tetrazol (metrazol) and electroshock in mice and their modification by anticonvulsants. J. Pharmacol. Exp. Ther. 108, 168–176.

Holmes, B.B., Fujimoto, J.M., 1994. [D-Pen^2-D-Pen^5]enkephalin, a delta opioid agonist, given intracerebroventricularly in the mouse produces antinociception through mediation of spinal GABA receptors. Pharmacol. Biochem. Behav. 49, 675–682.

Horváth, K., Andrási, F., Berzsenyi, P., Pátfalusi, M., Patthy, M., Szabó, G., Sebestyén, L., Bagdy, E., Botka, P., Hámori, T., Láng, T., 1989. A new psychoactive 5H-2,3-benzodiazepine with unique spectrum of activity. Arzneimittelforschung 39, 894–899.

Horváth, K., Andrási, F., Botka, P., Hámori, T., 1992a. Anxiolytic profile of girisopam and GYKI 52 322 (EGIS 6775). Comparison with chlordiazepoxide and buspirone. Acta Physiol. Acad. Sci. Hung. 79, 153–161.

Horváth, K., Szentkuti, E., Andrási, F., 1992b. Girisopam and GYKI 52 322 do not potentiate the anticonvulsant and antiaggressive effect of chlordiazepoxide in mice. Pharmacol. Res. 25 (Suppl. 2), 41–42.

Horváth, E.J., Hudák, J., Palkovits, M., Lenkei, Z., Fekete, M.I.K., Arányi, P., 1993. A novel specific binding site for homophtalazines in the rat brain. Eur. J. Pharmacol. 236, 151–153.

Horváth, E.J., Palkovits, M., Lenkei, Z., Gyüre, K.I., Fekete, M.I.K., Arányi, P., 1994. Autoradiographic localization and quantitative determination of specific binding sites of anxiolytic homophtalazines (formerly called 2,3-benzodiazepines) in striato-pallido-nigral system of rats. Mol. Brain Res. 22, 211–218.

Johnston, G.A., 1992. GABA A agonists as targets for drug development. Clin. Exp. Pharmacol. Physiol. 19, 73–78.

Kendall, D.A., Browner, M., Enna, S.J., 1982. Comparison of the antinociceptive effect of gamma-aminobutyric acid (GABA) agonists: Evidence for cholinergic involvement. J. Pharmacol. Exp. Ther. 220, 482–487.

Kenessey, Á., Gráf, L., Páldi-Haris, P., Láng, T., 1987. Interaction of 2,3-benzodiazepines with peripheral benzodiazepine receptors. Pharmacol. Res. Commun. 19, 1–14.

Koch, J.E., Glass, M.J., Cooper, M.L., Bodnar, R.J., 1995. Alterations in deprivation, glucoprivic and sucrose intake following general, mu and kappa oipioid antagonists in the hypothalamic paraventricular nucleus of rats. Neurosci. 66, 951–957.

Kőrösi, J., Láng, T., 1971. Heterocyklische Verbindungen I. Synthese des ersten 5H-2,3-Benzodiazepines, des 5-Äthyl-1-(3,4-dimethoxyphenyl)-7,8-dimethoxy-4-methyl-5H-2,3-benzodiazepins. Chem. Ber. 107, 3883–3907.

Kőrösi, J., Láng, T., Székely, J.I., Andrási, F., Zólyomi, G., Borsy, J., Horváth, K., Hámori, T., Czibula, G., Dunai-Kovács, Z., Miglécz, E., 1981. Procedure for synthesis of new 5H-2,3-benzodiazepine derivatives. Patent, HU 179.018 (1980); UK 2,034.706 (1980); USA 4,322.346 (1981).

Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Massi, P., Rubino, T., Parenti, M., Giagnoni, G., Gropetti, A., Parolaro, D., 1993. Cholera toxin antagonizes morphine-induced catalepsy through a cyclic AMP-independent mechanism. Eur. J. Pharmacol. 243, 65–71.

Paakkari, P., Feuerstein, G., 1988. Antagonism of dermorphin-induced catalepsy with naloxone, TRH-analog CG3703 and the benzodiazepine antagonist, RO 15-1788. Neuropharmacology 27, 1007–1012.

Páldi-Haris, P., Gráf, L., Kenessey, Á., Láng, T., 1985. Enhancement of low affinity GABA binding by diazepam and a new 2,3-benzodiazepine. Eur. J. Pharmacol. 109, 305–306.

Pellow, S., File, S.E., 1986. Is tofisopam an atypical anxiolytic?. Neurosci. Biobehav. Rev. 10, 221–227.

Rattan, A.K., Sribanditmonghol, P., 1994. Effect of morphine-induced catalepsy, lethality and analgesia by a benzodiazepine receptor agonist imidazolin in the rat. Pharmacol. Biochem. Behav. 48, 357–361.

Rondeau, D.B., Turcotte, M., Young, L., Herbert, D., 1982. Similarities

of the cataleptic state induced by beta-endorphin and morphine. Pharmacol. Biochem. Behav. 17, 703–707.

Rocha, L., Tatsukawa, K., Chugani, J. Jr., 1993. Benzodiazepine receptor binding following chronic treatment with naloxone, morphine and met-enkephalin in normal rats. Brain Res. 612, 247–252.

Saano, V., Urtti, A., 1982. Tofisopam modulates the affinity of benzodiazepine receptors in rat brain. Pharmacol. Biochem. Behav. 17, 367–369.

Salamon, C., Horváth, E.J., Fekete, M.I.K., Arányi, P., 1992. A novel specific binding site for anxiolytic homophtalazines in the rat brain. FEBS Lett. 308, 215–217.

Sawynok, J., 1987. GABAergic mechanisms of analgesia: An update. Pharmacol. Biochem. Behav. 26, 463–474.

Sivam, S.P., Ho, K.I., 1983. GABAergic drugs, morphine and morphine tolerance: A study in relation to nociception and gastrointestinal transit in mice. Neuropharmacology 22, 767–774.

Sivam, S.P., Ho, K.I., 1985. GABA in morphine analgesia and tolerance. Life Sci. 37, 199–208.

Swinyard, E.A., Brown, W.C., Goodman, L.S., 1952. Comparative assays of antiepileptic drugs in mice and rats. J. Pharmacol. Exp. Ther. 106, 319–330.

Tedeschi, R.E., Tedeschi, D.H., Mucha, A., Cook, L., Mattis, P.A., Fellows, E.J., 1959. Effects of various centrally acting drugs on fighting behaviour of mice. J. Pharmacol. Exp. Ther. 125, 28–34.

Zarrindast, M.R., Zarghi, A., 1992. Morphine stimulates locomotor activity by an indirect dopaminergic mechanism: Possible D-1 and D-2 receptor involvement. Gen. Pharmacol. 23, 1134–1221.