Pharmacology Biochemistry and Behavior, Vol. 65, No. 3, pp. 465–474, 2000

0091-3057/00/$–see front matter

PII S0091-3057(99)00228-2

Anxiolytic-Like Effects of Meprobamate: Interactions With an Opiate Antagonist in Swiss and BALB/c Mice

CATHERINE BELZUNG,* ANNE MARIE LE GUISQUET* AND ANDERS ÅGMO†

*Laboratoire d'Ethologie et Pharmacologie du Comportement, Faculté des Sciences, Université de Tours, Tours, France; and †Department of Psychology, University of Tromsø, Tromsø, Norway

Received 6 June 1999; Revised 7 September 1999; Accepted 21 September 1999

BELZUNG, C., A. M. LE GUISQUET AND A. ÅGMO. *Anxiolytic-like effects of meprobamate: Interactions with an opiate antagonist in Swiss and BALB/c mice.* PHARMACOL BIOCHEM BEHAV **65**(3) 465–474, 2000.—Naloxone has previously been shown to block the effects of benzodiazepines in the Swiss but not in the BALB/c strain. We have also reported that naloxone potentiates subeffective doses of benzodiazepines in Swiss mice. In the present studies we first determined whether naloxone could block anxiolytic-like effects of meprobamate in Swiss and BALB/c mice. Then we evaluated if subeffective doses of meprobamate could be potentiated in Swiss as well as in BALB/c mice. The elevated plus-maze test and the light/dark choice procedure were used. The lowest dose of meprobamate with anxiolytic-like effects was 60 mg/kg in the BALB/c mice. This dose was effective in both the plus-maze and in the light/dark choice procedure. In Swiss mice the same dose was effective in the plus-maze, whereas 120 mg/kg was required in the light/dark choice procedure. When an effective dose of meprobamate was combined with naloxone, 10 mg/kg, no blockade of anxiolytic-like effects was obtained in any strain in any procedure. To the contrary, when a subeffective dose of meprobamate was combined with naloxone, 10 mg/kg, an anxiolytic-like effect was obtained in both strains in both procedures. The present series of experiment shows that the ability of naloxone to block anxiolytic-like drug effects do not apply to meprobamate. However, the naloxone-induced potentiation of subeffective doses previously observed after treatment with benzodiazepines or buspirone was present also after treatment with meprobamate. Moreover, although blockade of anxiolytic-like drug effects with naloxone has not been observed in BALB/c mice, potentiation was as evident in that strain as in the Swiss. This suggests that the mechanisms behind naloxone's blockade of anxiolytic-like effects are independent from those behind its potentiation of such effects. © 2000 Elsevier Science Inc.

Meprobamate BALB/c mice Swiss mice Plus-maze test Light/dark choice procedure Anxiolytic Opiates

THERE is much evidence showing that anxiolytic-like effects of benzodiazepines can be blocked by the opiate antagonist naloxone in humans (26), rats (11,25,38,66) and mice (2,6,73). Furthermore, the anxiolytic effects of barbiturates are blocked by naloxone (4). However, the motor relaxant effects of benzodiazepines and barbiturates are not reduced by the opiate antagonist (4,73). These observations suggest that opioid systems in some way or another are involved in the anxiolytic action of drugs supposed to act at the supramolecular $GABA_A$ receptor complex but not in the motor actions of these drugs. We have proposed that opioid systems need to be activated in order for benzodiazepines or barbiturates to display anxiolytic effects (1,4). In support of that hypothesis we have reported that naloxone does not block the anxiolytic-like effects of diazepam or chlordiazepoxide in BALB/c mice (2,6). These mice are known to be highly "emotional" in most stress-inducing environments. They show intense neophobia (31,54,69,70), strong avoidance of the lit area in the light/dark choice procedure (31,49), and of the open arm on the elevated plus-maze (2,6). Moreover, they release more corticosteroids in response to stress than other mouse strains (63). However, in nonstressful situations, like a dark open field or a plus-maze under low ambient light BALB/c mice may even be less "emotional" than other strains (72). It has been re-

Requests for reprints should be addressed to Anders Ågmo, Institutt for Psykologi, Universitetet i Tromsø, N-9037 Tromsø, Norway.

ported that these mice show a deficient opioid response to stress (17,20,48), and this may account both for their emotionality and for the inability of naloxone to antagonize the anxiolytic effects of benzodiazepines. The notion that opioid release reduces the emotional impact of stressors is supported by studies in the human where a negative correlation between subjective stress and the amount of opioids released in response to stress has been found (13,23).

In contrast to the above-mentioned observations, there are also data showing that subeffective doses of anxiolytic agents can be potentiated by naloxone in the elevated plus-maze test (7). This was the case for the benzodiazepines chlordiazepoxide and diazepam as well as for the partial 5-HT_{1A} agonist buspirone. Interestingly, while the anxiolytic-like effects of the drugs were potentiated by naloxone, their motor effects were not. The effective doses of naloxone are the same for the blockade and potentiation of anxiolytic-like effects (1,2,6,7). This fact is difficult to explain, but it is possible that the mechanisms behind potentiation of anxiolytic effects are different from those involved in its blockade. One possibility is that different opioid receptors are involved. It has been reported that the μ and κ receptors are important for the blockade of anxiolytic-like effects (1,73). Perhaps the δ receptor is material to the potentiation. Another explanation is that blockade of opioid receptors with naloxone removes inhibitory opioidergic influences on GABA neurons, thereby reinforcing the effects of low doses of benzodiazepines. Similarly, opioid/GABA interactions within the nucleus raphe may account for the potentiation of buspirone (7).

In an effort to analyze the opposing interactions between opioid receptors and anxiolytic-like drug effects we decided to test a nonbenzodiazepine anxiolytic, the propanediol carbamate meprobamate. This drug has effects very similar to those of benzodiazepines in rats and mice in several behavioral paradigms, for example, the staircase test (59,64), different versions of the Geller–Seifter procedure (29,34,61), place-preference conditioning (68), marble burying and grooming (14), food or water consumption in novel and familiar environments (65,67), intracerebral self-stimulation (16,43), passive avoidance learning (42), a kind of light/dark choice test (21) and in the Vogel procedure (74). However, its mechanism of action is probably different from that of the benzodiazepines. The benzodiazepine antagonist flumazenil does not inhibit the effects of meprobamate (12,52). Moreover, meprobamate affects the chloride ionophore in a way similar to that of benzodiazepines only at very high concentrations (45). The drug inhibits diazepam binding, but again, at high concentrations (53). It is likely, therefore, that at least part of the actions of meprobamate is independent of the GABA/benzodiazepine receptor.

With the purpose of further analyzing the complex interactions of the opioid system with anxiolytic drugs, we decided to determine if naloxone could block the actions of effective doses of meprobamate in Swiss and BALB/c mice in the elevated plus-maze and the light/dark choice tests. As mentioned above, naloxone does not block the effects of chlordiazepoxide in the BALB/c strain (2,6), and it seemed worthwhile to determine if this was also the case for meprobamate. In a further series of experiments we administered subeffective doses of meprobamate together with naloxone to evaluate if anxiolytic-like effects of this drug could be potentiated in Swiss and BALB/c mice. If it were in both strains, then we could conclude that the mechanisms behind potentiation are different from those related to blockade. Otherwise, no potentiation would have occurred in the BALB/c mice.

METHOD

Subjects

Male Swiss and BALB/c ByJICo mice were obtained from Janvier (Le Genest Saint Isle) when 7 weeks old. All subjects were housed five per standard cage at a constant temperature of about 22°C under a reversed light/dark cycle (12/12 h, lights off at 0800 h). Mice are nocturnal animals with peak activity during the first and last few hours of the dark period. During the middle part of the dark period activity is at a stable level, higher than during the light period [e.g., (41)]. Because both the light/dark choice procedure and the elevated plus-maze are considered to be ethologically relevant, it seems natural to test the animals at a time when they normally would be active. The phase shift of locomotor activity and most endocrine rhythms is complete within 2 weeks (39,75), i.e., before experiments were initiated. Commercial rodent pellets and tap water were freely available. The treatment of the animals was in accordance with the European Community Council directive 86/609/EEC. Experiments were begun about 3 weeks after the animals arrived to the laboratory.

Apparatus and Procedure

Elevated plus-maze. The mazes, made of yellowish polyvinylchloride, were elevated to a height of 38.5 cm and placed in a dark room. The open arm (59 × 5 cm) were lit by 60-W transparent bulbs hanging 50 cm above each end. Light intensity on the arm's surface was about 550 lx. The closed arm (59 × 5 cm) had 15 cm-high walls (also made of polyvinylchloride), and was covered with dark paper during the tests. At the intersection of the arms there was an open platform measuring 5 × 5 cm. This experimental setup assured a low proportion of entries onto the open arm, and has been shown to reliably detect anxiolytic drug effects with a minimum confound of drug actions on ambulatory activity (1,2,6,7).

Tests began with the mouse being placed on the central platform with its head facing the open arm. Arm entries were registered on a hand-held computer (Psion Organiser) for 5 min. The mouse was considered to be on an arm when the four paws were on it. Consequently, it was considered to be on the central platform whenever it had at least one paw on it.

Light/dark choice procedure. The apparatus consisted of two boxes (20 × 20 × 14 cm high) connected with a tunnel (10 × 7 × 5 cm high). One box and the tunnel were made of opaque polyvinylchloride, whereas the other box was made of transparent Plexiglas. A desk lamp with a 100-W bulb placed about 20 cm above the Plexiglas lid provided intense illumination.

At the beginning of a test the mouse was placed in the middle of the light compartment with its head facing the tunnel. Behavioral recording started when the subject had entered the tunnel with its four paws and continued for 5 min thereafter. The number of transitions between compartments and the time spent in the lit compartment were registered with the help of a Psion Organiser.

All tests were performed between the sixth and the ninth hour of the dark phase.

Design

A parallel groups design was used, i.e., all doses of a drug and/or combinations of drugs included in a particular experiment were run at a single session in each strain. The order of drug treatments within a session was counterbalanced. There

were 8 to 14 mice per group, and they were all experimentally naive.

Drugs

A commercial preparation of meprobamate (Equanil®) was used. The solution was diluted to appropriate concentration with hot distilled water. At the time of injection, the solution was at room temperature. Naloxone HCl was purchased from Sigma (St. Louis, MO) and dissolved in physiological saline. Saline was also injected into control animals. The drugs were injected intraperitoneally in a volume of 1 ml/100 g body weight. Meprobamate was administered 30 min before test and naloxone 15 min before. Drug doses refer to the form of the compound stated above.

Statistics

The total number of arm entries (a measure of ambulatory activity) and the proportion of entries on the open arm (number of open arm entries/total number of entries, a measure of anxiolytic-like effects) as well as the number of transitions and the time spent in the lit box were analyzed with the nonparametric Kruskall–Wallis ANOVA. A posteriori comparisons were made with the Mann–Whitney *U*-test. Parametric tests were not employed because of a nonnormal distribution of the data and nonhomogenous error variances in some cases. It may be noted that most of the saline-treated BALB/c mice made 0 entries on the open arm on the plus-maze and remained in the tunnel during the entire test in the light/dark choice, procedure thereby assuring a highly skewed distribution. Although data from the Swiss mice could have been analyzed with parametric tests, we considered it important to use the same analysis for all data. Probabilities given are always two tailed.

RESULTS

Effects of Meprobamate

As shown in Fig. 1, a meprobamate dose of 30 mg/kg was ineffective while a dose of 60 mg/kg was sufficient to increase the proportion of open arm entries in both strains. A larger dose, 120 mg/kg, also increased the proportion of open-arm entries and, in the BALB/c strain, the total number of entries as well.

Dose–effect relationships were somewhat more complex in the light/dark choice test. Here, a dose of 30 mg/kg enhanced the number of transitions between the light and the dark compartment in the Swiss strain without modifying the time spent in the lit compartment. A dose of 60 mg/kg increased the number of transitions in both strains. It also increased the time spent in the lit compartment in the BALB/c strain. This time was increased in both strains at a dose of 120 mg/kg. The number of transitions was only increased in the BALB/c mice at this dose. As in the procedure employed, an anxiolytic effect corresponds to an increase in the time spent in the lit box the minimum effective dose was 60 mg/kg in the BALB/c and 120 mg/kg in the Swiss mice. Data are illustrated in Fig. 2.

Effects of Naloxone

Complete dose–effect curves for the interaction between naloxone and benzodiazepines have been reported previously (4). The 10 mg/kg dose used in the present studies efficiently antagonizes the effects of suprathreshold doses of diazepam, chlordiazepoxide, and pentobarbital. As can be seen in Figs. 3 and 4, this dose of naloxone had no intrinsic anxiolytic or anxiogenic activity. However, in the elevated plus-maze it tended to decrease the total number of arm entries in Swiss mice (Fig. 3A). The effect was of borderline significance ($p = 0.051$).

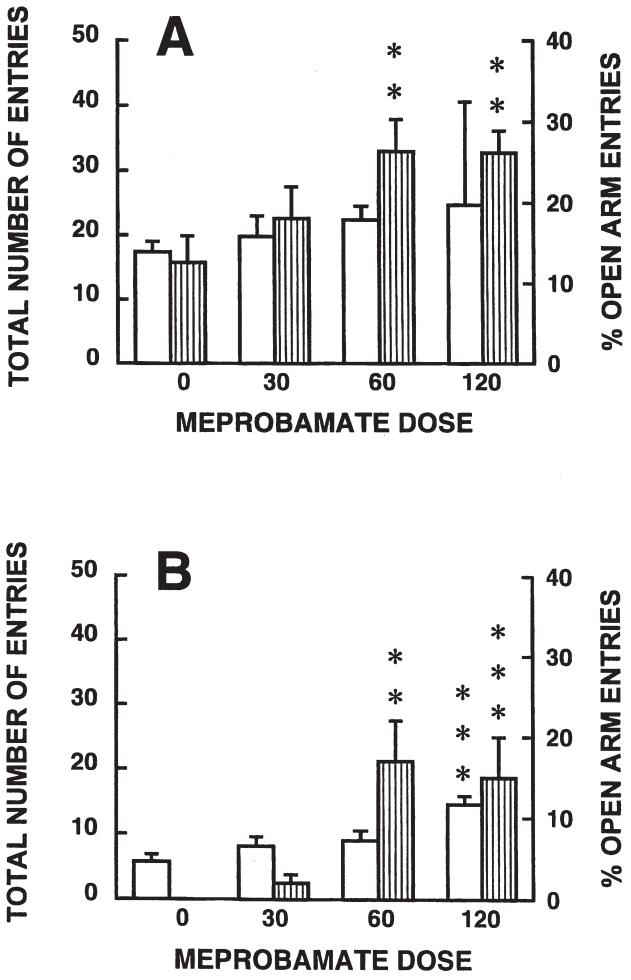

FIG. 1. Total number of arm entries (open bars; left ordinate) and (number of entries onto the open arm/total number of entries) × 100 (striped bars, right ordinate) in Swiss (A) and BALB/c (B) mice treated with varying doses of meprobamate and tested on the elevated plus-maze. Data are mean + SEM. The meprobamate dose is expressed in mg/kg. Kruskal–Wallis H was 5.76, NS for the Swiss mice, and 14.94, $p = 0.002$ for the BALB/c mice with regard to the total number of arm entries and 9.61, $p = 0.02$ for the Swiss mice, and 15.78, $p < 0.001$ for the BALB/c with regard to the proportion of open arm entries. **Different from control, $p < 0.01$; ***$p < 0.001$ as determined by the Mann–Whitney *U*-test.

Naloxone Combined With an Effective Dose of Meprobamate

When the least effective dose of meprobamate was combined with naloxone, 10 mg/kg, in the elevated plus-maze test there was no inhibition of anxiolytic-like effects. To the contrary, the effect of meprobamate, 60 mg/kg, + naloxone was significantly larger than the effect of meprobamate + saline in the BALB/c strain. In this experiment meprobamate increased the total number of arm entries in both strains, an ef-

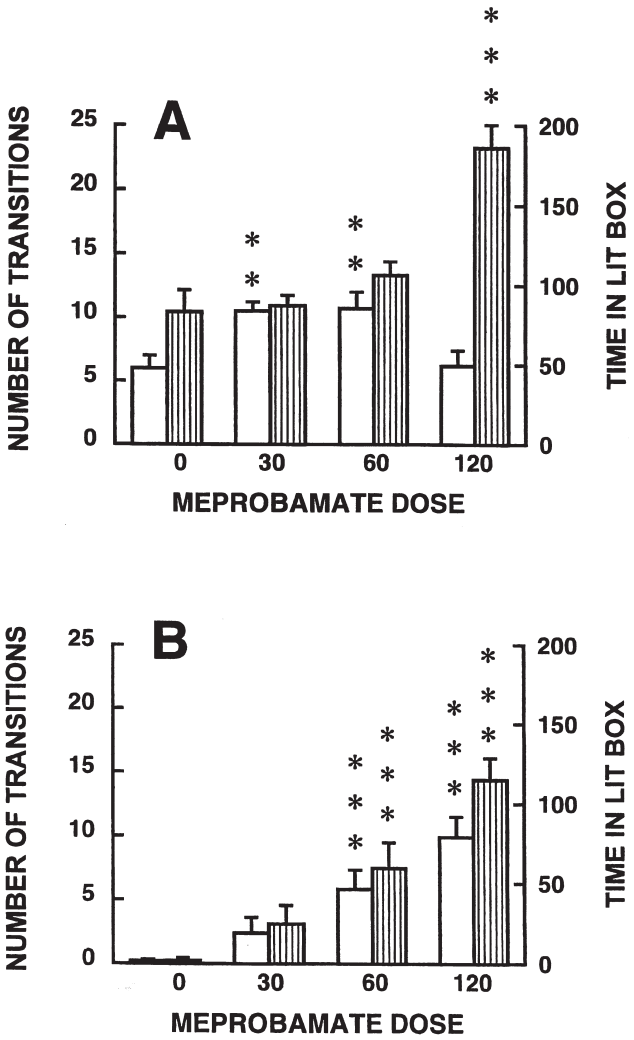

FIG. 2. Number of transitions between boxes (open bars, left axis) and the time spent in the lit box (striped bars, right axis) in Swiss (A) and BALB/c (B) mice treated with varying doses of meprobamate and tested in the light/dark choice procedure. Data are mean + SEM. The meprobamate dose is expressed in mg/kg and the time spent in the lit box is in seconds. Kruskal–Wallis H was 13.21, $p = 0.004$ for the Swiss and 24.60, $p < 0.001$ for the BALB/c mice with regard to the number of transitions and 22.59, $p < 0.001$ for the Swiss mice and 26.37, $p < 0.001$ for the BALB/c with regard to the time spent in the lit compartment. **Different from control, $p < 0.01$; ***$p < 0.001$ as determined by the Mann.–Whitney U-test.

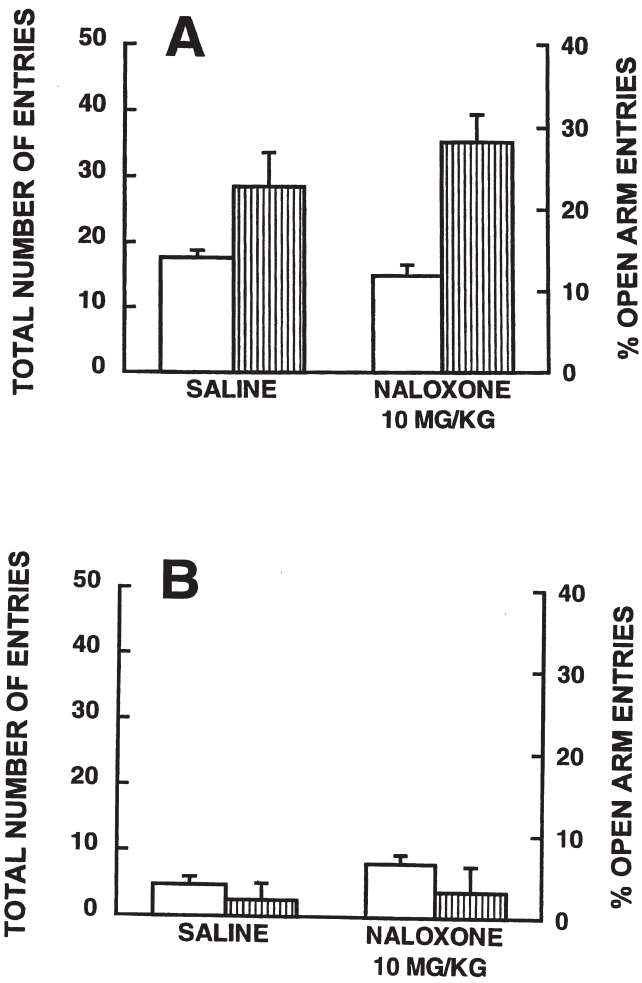

FIG. 3. Total number of arm entries (open bars; left ordinate) and (number of entries onto the open arm/total number of entries) × 100 (striped bars, right ordinate) in Swiss (A) and BALB/c (B) mice treated with naloxone, 10 mg/kg, and tested on the elevated plus-maze. For further details, see text to Fig. 1. The Mann–Whitney U statistic for the total number of entries was 50.5, $p = 0.051$ for the Swiss and 20.0, NS for the BALB/c mice. With regard to the proportion of open arm entries corresponding values were 19.5, NS, and 41.0, NS, respectively.

fect that was not seen in the first experiment reported above. Interestingly, this increase was blocked by naloxone in both strains (see Fig. 5). None of them differed from control on this parameter when meprobamate was combined with naloxone, and in the BALB/c strain the difference between meprobamate + saline and meprobamate + naloxone was significant. It seems, then, that naloxone is unable to block meprobamate's anxiolytic-like effects while its motor stimulating actions are reduced. These observations were not confirmed in the light/dark choice procedure. Just as in the plus-maze, naloxone did not block the increase of the time spent in the lit box produced by meprobamate in any strain. Again, the effect of meprobamate + naloxone was significantly larger than that of meprobamate + saline in the BALB/c strain. However, the meprobamate-induced increase in the number of transitions between boxes was blocked in Swiss but not in BALB/c mice. Data are summarized in Figure 6.

Naloxone Combined With a Subeffective Dose of Meprobamate

On the elevated plus-maze a dose of meprobamate that had no effect by itself (30 mg/kg) on the proportion of open arm entries was potentiated by naloxone, 10 mg/kg, in both strains. In fact, the proportion of open arm entries was enhanced when compared to control, and also when compared to meprobamate + saline in the BALB/c strain. There was no effect on the total number of entries. Data are shown in Fig. 7.

In the light/dark choice test, meprobamate, 30 mg/kg, was also potentiated by naloxone in both strains. Indeed, mep-

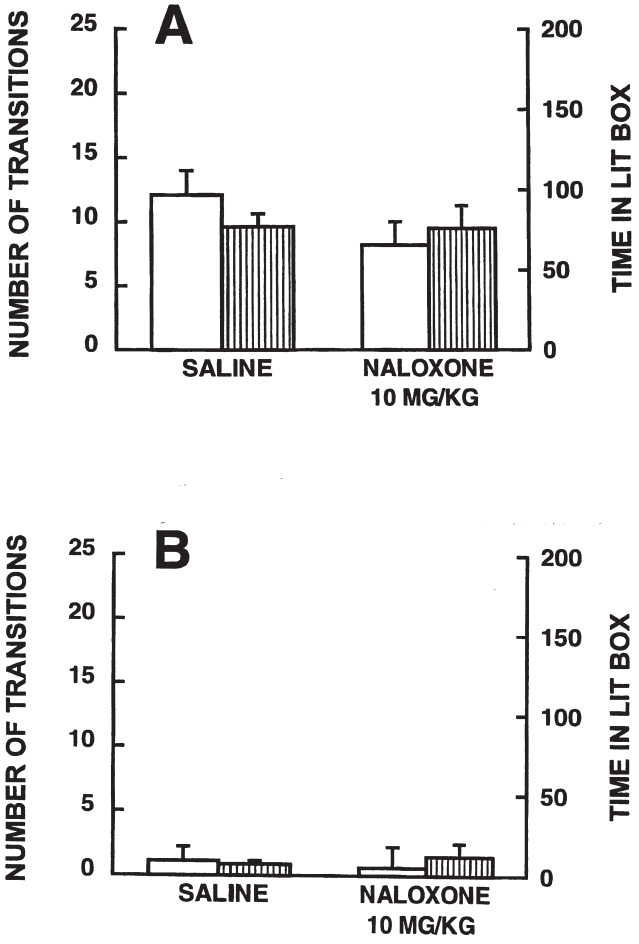

FIG. 4. Number of transitions between boxes (open bars, left axis) and the time spent in the lit box (striped bars, right axis) in Swiss (A) and BALB/c (B) mice treated with naloxone, 10 mg/kg, and tested in the light/dark choice procedure. The Mann–Whitney *U* statistic for the number of transitions was 109, NS, for the Swiss, and 74, NS, for the BALB/c mice. Corresponding values for the time spent in the lit compartment were 76, NS, and 71, NS, respectively.

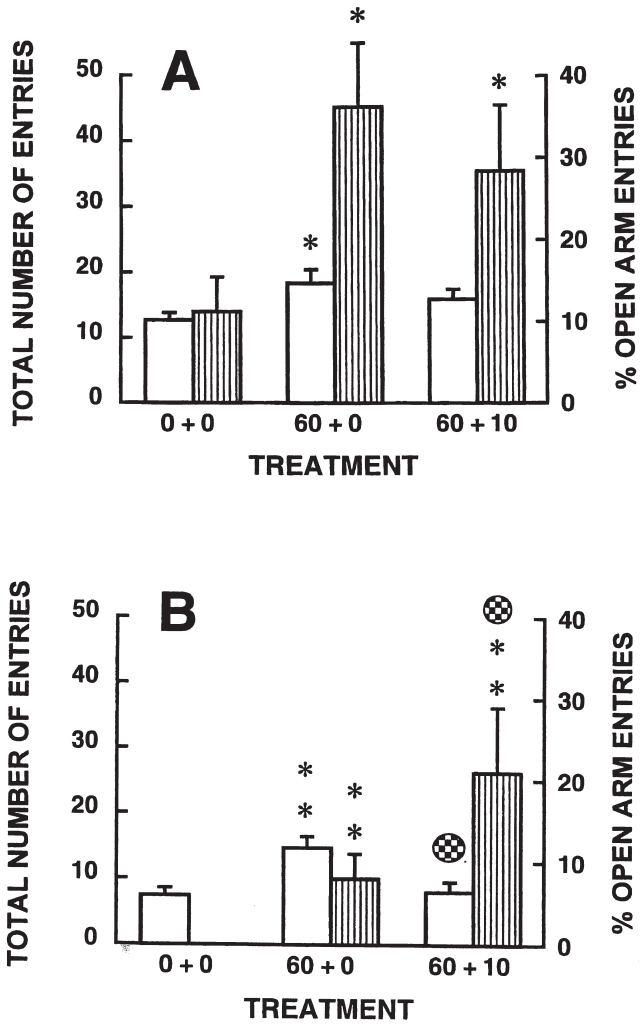

FIG. 5. Total number of arm entries (open bars; left ordinate) and % open arm entries (striped bars, right ordinate) in Swiss (A) and BALB/c (B) mice treated with the least effective dose of meprobamate in combination with naloxone and tested on the elevated plus-maze. The meprobamate dose (in mg/kg) is given to the left of the + sign and the naloxone dose to the right. For further details, see Fig. 1. Kruskal–Wallis H was 6.47, $p = 0.039$ for the Swiss mice and 9.44, $p = 0.009$ for the BALB/c with regard to the total number of arm entries and 6.71, $p = 0.035$ for the Swiss and 10.07, $p = 0.006$ for the BALB/c with regard to the proportion of open arm entries. *Different from control, $p < 0.05$; **$p < 0.01$. Patched circle is different from meprobamate 60 mg/kg + saline, $p < 0.01$ according to the Mann–Whitney *U*-test.

robamate + naloxone was not only significantly different from control but also from meprobamate + saline in both strains. In contrast to the elevated plus-maze, ambulatory activity was also increased by the combination of drugs (Fig. 8). As in the dose–effect experiment reported above, meprobamate + saline augmented the number of transitions in Swiss mice. This effect was not modified by the addition of naloxone.

DISCUSSION

Present data show that meprobamate is an efficient anxiolytic in Swiss and BALB/c mice. This is not surprising, because the drug has been reported to have robust anxiolytic-like effects in rats of several strains and in several procedures (14,29,34,52,59,61,64,74). The doses needed for significant effects in those studies (30–128 mg/kg) are similar to those needed in the present ones. It is most unlikely that the increase in the proportion of open-arm entries and in the time spent in the lit box can be explained solely as a consequence of drug-induced stimulation of ambulatory activity. In the Swiss strain, there was a clear-cut separation between effects on the parameters supposed to reflect anxiolytic-like actions and on those reflecting ambulatory activity, i.e., the total number of arm entries on the plus-maze and the number of transitions in the light/dark choice procedure. Such a clear-cut separation between effects on ambulatory activity and on anxiolytic-like effects was not obtained in the BALB/c strain. Here, effects on parameters sensitive to anxiolytic-like effects were frequently, but not always, associated with effects on parameters sensitive to effects on ambulatory activity. The ex-

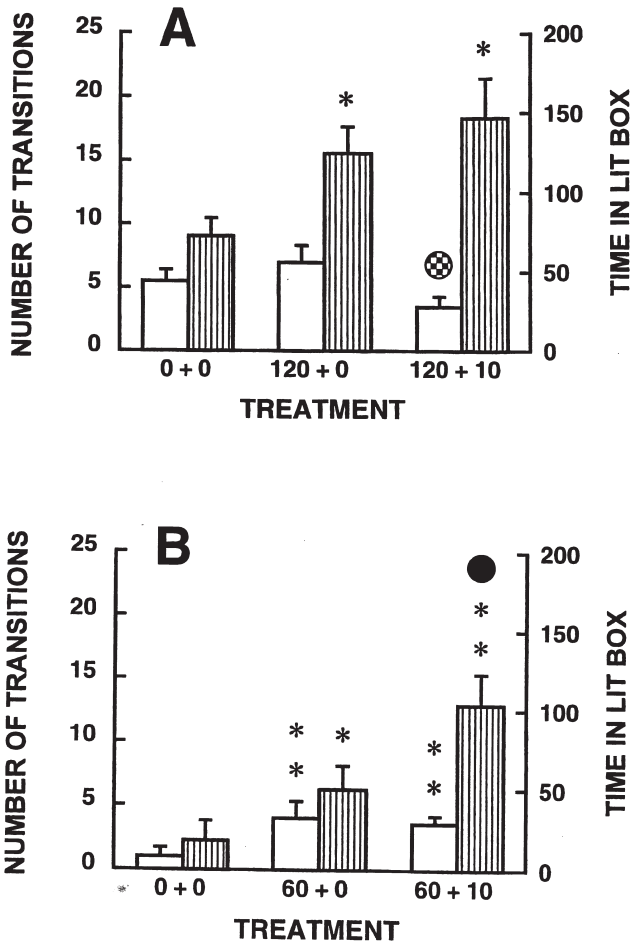

FIG. 6. Number of transitions between boxes (open bars, left axis) and the time spent in the lit box (striped bars, right axis) in Swiss (A) and BALB/c (B) mice treated with the least effective dose of meprobamate in combination with naloxone and tested in the light/dark choice procedure. For further details, see Fig. 2. Kruskal–Wallis H was 4.57, NS for the Swiss mice and 9.47, $p = 0.009$ for the BALB/c with regard to the number of transitions and 8.07, $p = 0.02$ for the Swiss mice and 14.58, $p = 0.001$ for the BALB/c with regard to the time spent in the lit box. *Different from control, $p < 0.05$; **$p < 0.01$. Patched circle is different from meprobamate 120 mg/kg + saline, $p < 0.05$; Closed circle is different from 60 mg/kg, $p < 0.01$ as evaluated with the Mann–Whitney U-test.

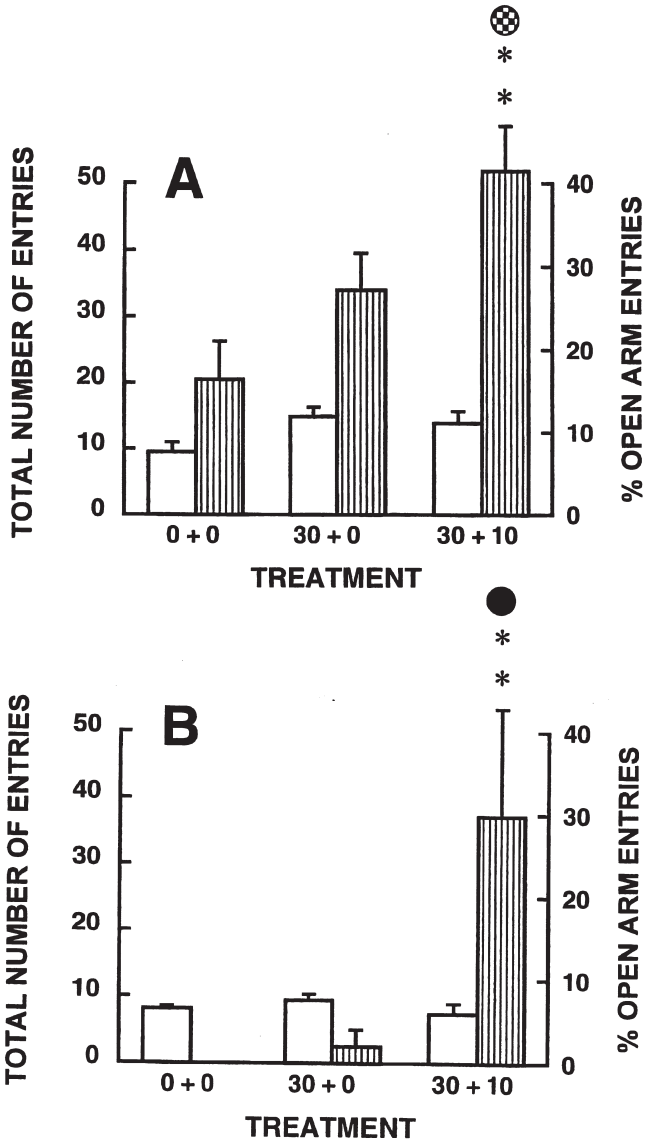

FIG. 7. Total number of arm entries (open bars; left ordinate) and % open arm entries (striped bars, right ordinate) in Swiss (A) and BALB/c (B) mice treated with a subeffective dose of meprobamate in combination with naloxone and tested on the elevated plus-maze. For further details, see Fig. 3. Kruskal–Wallis H was 5.19, NS, for the Swiss mice and 2.78, NS, for the BALB/c mice with regard to the total number of arm entries and 9.47, $p < 0.009$ for the Swiss mice and 9.93, $p = 0.007$ for the BALB/c with regard to the proportion of open arm entries. **Different from control, $p < 0.01$. Patched circle is different from meprobamate 30 mg/kg + saline, $p < 0.05$; Closed circle, $p < 0.01$ according to the U-test.

ception was meprobamate, 60 mg/kg, in the plus-maze, where the proportion of open-arm entries was increased without any concurrent effect on the total number of arm entries. One explanation for the association between anxiolytic-like and locomotor stimulating effects is that the BALB/cs' high emotionality (see introduction) has a suppressing effect on all kinds of exploratory behaviors in novel, stress-inducing environments, and that anxiolytic drugs relieve that suppression. The brightly lit plus-mazes and light/dark choice apparatuses used here can certainly be considered stress inducing, as shown by the fact that the BALB/c mice spent no time on the open arm of the plus-maze and very little in the light box after saline treatment.

Naloxone had no intrinsic anxiolytic-like effect in these procedures, in agreement with some previous reports (2,40). There are studies, though, where naloxone at a dose of 5 mg/kg has been found to have an anxiolytic-like action in mice, tested in the light/dark choice procedure (6,51). In the latter study, no larger dose was tested, but in the former one a dose of 10 mg/kg was ineffective. It is possible that naloxone has an inverted U-shaped dose–effect curve in this procedure. Nevertheless, the effect was of small magnitude in both studies, and it is most unlikely that it could account for the effects obtained in the present studies.

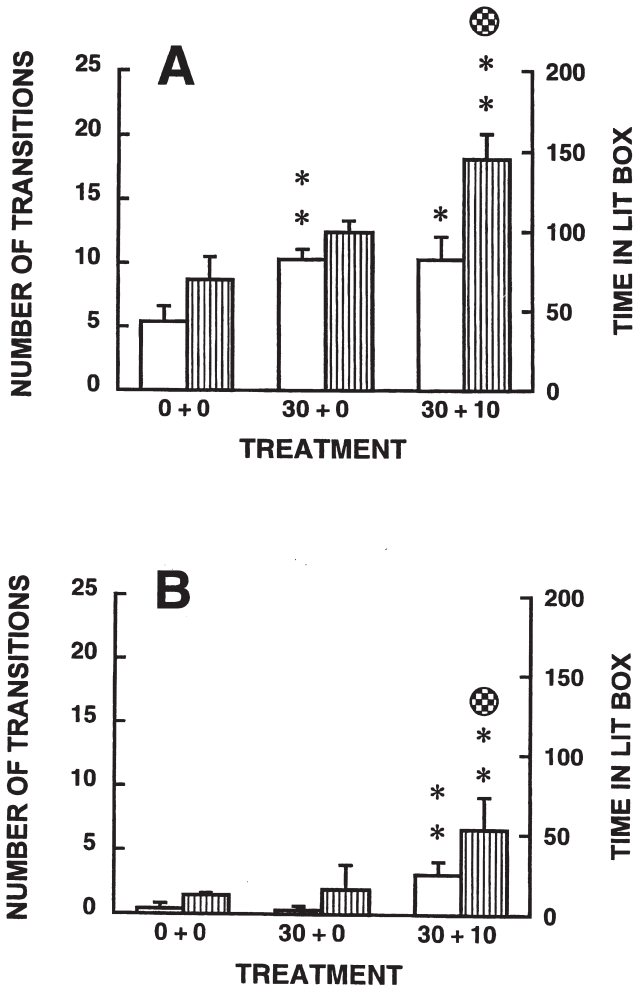

FIG. 8. Number of transitions between boxes (open bars, left axis) and the time spent in the lit box (striped bars, right axis) in Swiss (A) and BALB/c (B) mice treated with a subeffective dose of meprobamate in combination with naloxone and tested in the light/dark choice procedure. For further details, see Fig. 4. Kruskal–Wallis H was 9.36, $p = 0.009$ for the Swiss mice and 10.38, $p = 0.006$ for the BALB/c with regard to the number of transitions and 11.26, $p = 0.004$ for the Swiss mice and 9.19, $p = 0.01$ for the BALB/c with regard to the time spent in the lit compartment. *Different from control, $p < 0.05$; **$p < 0.01$. Patched circle is different from meprobamate 30 mg/kg + saline, $p < 0.01$ as determined by the U-test.

Naloxone did not block or even reduce the effects of meprobamate in any strain in any procedure. In fact, the opposite effect was obtained in the BALB/c strain. Here, naloxone enhanced the anxiolytic-like effects of meprobamate both in the elevated plus-maze and in the light/dark choice procedure. It might be argued that the lack of inhibitory effect of naloxone is a consequence of an inadequate dose. However, the dose employed in the present study has been shown to effectively block the anxiolytic-like effects of benzodiazepines in mice and rats (2,4,6). It can, therefore, be concluded that opioid systems are not essential for the actions of meprobamate, although they seem to be so for the anxiolytic-like effects of drugs acting at the GABA/benzodiazepine receptor (2,4,6). In this context it could be mentioned that preliminary studies from our laboratories have shown that naloxone does not block anxiolytic-like effects of buspirone (9). It has also been reported that naloxone fails to block anxiolytic effects of alcohol in rats tested on the plus-maze (5). It is possible, then, that opioid receptor antagonists interfere with the anxiolytic effects of drugs acting at the GABA/benzodiazepine receptor but not with those of drugs acting elsewhere.

It could be argued that the ability of naloxone to block the anxiolytic action of drugs interacting with the $GABA_A$ receptor is due to naloxone's purported GABA antagonist properties (24). These properties were inferred from data showing that extremely large doses of naloxone induced convulsion and high concentrations reduced GABA binding in vitro. However, later studies have shown that blockade of the opioid system induces convulsions (27,37,55), and the former effect may, therefore, be due to naloxone's opioid antagonist action. Furthermore, there are more recent data demonstrating that naloxone does not bind to the $GABA_A$ receptor to any significant degree (30). Affinity for the serotonin1_A receptor is also insignificant (46). It may also be observed that naloxone does not block the motor incoordination produced by benzodiazepines or pentobarbital, whereas this is readily blocked by GABA antagonists (3,4). It seems, therefore, that there is little reason to attribute the effects of naloxone observed in the present studies to blockade of $GABA_A$ receptors or of any other receptor involved in the regulation of anxiety-like behaviors.

We have repeatedly shown that naloxone does not reduce the anxiolytic effects of benzodiazepines in the BALB/c strain (2,7). The data obtained here with meprobamate coincide with these observations. However, naloxone not only failed to block the actions of meprobamate but also enhanced them. This latter effect was not obtained in the Swiss mice. Present data confirm the proposal that the role of opioid systems in anxiolytic processes is different in BALB/c and in Swiss mice. A complete explanation of this difference must await further studies.

The inability of naloxone to block the effects of meprobamate is at difference to a study in rats where naloxone efficiently reduced the anticonflict effect of meprobamate in the Vogel procedure (25). It is unlikely that species (rats vs. mice) or procedural (Vogel vs. plus-maze) differences can account for this contradiction. We have obtained very similar results in rats and mice using either the Vogel procedure or the elevated plus-maze with regard to antagonism of benzodiazepine effects by naloxone (2,4,6). Moreover, the doses used were within the same range in both studies. Nevertheless, it is not entirely impossible that subtle procedural differences could account for the discrepancy between present data and those of Duka et al. (25).

A subeffective dose of meprobamate was potentiated by naloxone in both strains. This coincides with earlier observations showing that subeffective doses of the benzodiazepine chlordiazepoxide as well as the 5-HT_{1A} agonist buspirone are potentiated by the opiate antagonist (7,8). With regard to the mechanism of action, we have previously proposed that removal of the opioidergic inhibition of GABAergic neurons (19,36,44) with naloxone would reinforce the stimulatory effects of benzodiazepines on these neurons (7). In the case of buspirone, we suggested a similar although indirect mechanism. It is not evident that the potentiation of meprobamate can be explained by such an hypotheses. Although the propanediol carbamate has behavioral effects most similar to those of the benzodiazepines (see introduction), it is unlikely that these effects are mediated by the GABA/benzodazepine receptor. There is, however, a report suggesting that mep-

robamate may act at the barbiturate binding site on the GABA/benzodiazepine receptor (60). The importance of this is difficult to determine, particularly because meprobamate and barbiturates do not always have similar effects (10,32).

It seems, therefore, that some other site of action common to benzodiazepines, meprobamate, and ideally also to buspirone is involved in the process of potentiation. One such site is the adenosine receptors. Just as is the case with benzodiazepines (15,47,56,58), meprobamate potentiates the depressant actions of adenosine at concentrations within the therapeutic range (57). It appears that both benzodiazepines and meprobamate inhibit adenosine uptake (18,22). There are recent data suggesting that A_1 receptor agonists have anxiolytic-like effects in the elevated plus-maze and in the light/dark choice test (28,35), and an inhibitor of adenosine uptake is anxiolytic in the former procedure (76). Activation of the A_1 receptor is negatively coupled to adenylylcyclase whereas opioids may stimulate or inhibit this enzyme (62). In case they stimulate adenylylcyclase in brain regions important for anxiolytic effects, they would have an action opposite to that of A_1 receptor stimulation. Blockade of opiate receptors would, therefore, remove the opposing opioid action, and that could potentiate activities at the A_1 receptor. This explanation could also account for the effects of buspirone, because 5-HT_{1A} agonists inhibit adenylylcyclase (33,50,71) in a way similar to that of A_1 agonists. Obviously, much work remains to be done before this hypothesis can be substantiated by experimental fact.

Although an increasing amount of data show that there are intricate relationships between the opioid systems and the actions of several kinds of anxiolytic drugs, there is no comprehensive and experimentally verified hypothesis able to explain the data. The multiple actions of opioids at the cellular level, including blockage of calcium entry and activation of potassium channels [see (62)], in addition to the already mentioned effects on adenylylcyclase make it extremely difficult to delimit possible mechanisms of action. Nevertheless, the potentiation of anxiolytic effects shown to occur after treatment with naloxone could have substantial clinical interest. So far, it seems that anxiolytic-like effects are potentiated but not motor or sedative effects. Thus, subeffective doses of benzodiazepines, buspirone, or meprobamate, with virtually no side effects, could be rendered effective anxiolytics through combination with naloxone or other opiate antagonists. Obviously, clinical trials are needed before this proposal can leave the stage of speculation.

ACKNOWLEDGEMENTS

Raymond Jegat built the apparatus.

REFERENCES

1. Ågmo, A.; Belzung, C.: The role of subtypes of the opioid receptor in the anxiolytic action of chlordiazepoxide. Neuropharmacology 37:223–232; 1998.
2. Ågmo, A.; Belzung, C.; Deloire, X.; Grassin, M.; Lewis, S.: Blockade of anxiolytic-like actions of chlordiazepoxide by naloxone in the elevated plus-maze: Comparisons between Swiss, C57BL/6 and BALB/c mice. Psychobiology 27:105–113; 1999.
3. Ågmo, A.; Fernández, H.: Benzodiazepine receptor ligands and sexual behavior in the male rat: The role of GABAergic mechanisms. Pharmacol. Biochem. Behav. 38:781–788; 1991.
4. Ågmo, A.; Galván, A.; Heredia, A.; Morales, M.: Naloxone blocks antianxiety but not the motor effects of benzodiazepines and pentobarbital: Experimental studies and literature review. Psychopharmacology (Berlin) 120:186–194; 1995.
5. Badia-Elder, N.; Stewart, R. B.; Froehlich, S. C.: Naloxone does not alter the anxiolytic effect of alcohol. Soc. Neurosci. Abstr. 24:579.4; 1998.
6. Belzung, C.; Ågmo, A.: Naloxone blocks anxiolytic-like effects of benzodiazepines in Swiss but not in BALB/c mice. Psychopharmacology (Berlin) 132:195–201; 1997a.
7. Belzung, C.; Ågmo, A.: Naloxone potentiates the effects of subeffective doses of anxiolytic agents in mice. Eur. J. Pharmacol. 323:133–136; 1997b.
8. Belzung, C.; Dubreuil, D.: Naloxone potentiates the anxiolytic but not the amnestic action of chlordiazepoxide in C57BL/6 mice. Behav. Pharmacol. 9:691–698; 1998.
9. Belzung, C.; Le Guisquet, A. M.: Different pattern of interaction of three anti-anxiety compounds (chlordiazepoxide, diazepam and buspirone) with naloxone. Behav. Pharmacol. 8:642; 1998.
10. Berger, F. M.: The pharmacological properties of 2-methyl-2-*n*-propyl-1,3-propanediol dicarbamate (Miltown), a new interneuronal blocking agent. J. Pharmacol. Exp. Ther. 112:413–423; 1954.
11. Billingsley, M. L.; Kubena, R. K.: The effects of naloxone and picrotoxin on the sedative and anticonflict effects of benzodiazepines. Life Sci. 22:897–906; 1978.
12. Bonetti, E. P.; Pieri, L.; Cumin, R.; Dschaffer, R.; Pieri, M.; Gamzu, E. R.; Muller, R. K.; Haefely, W.: Benzodiazepine antagonist Ro 15-1788: Neurological and behavioral effects. Psychopharmacology (Berlin) 78:8–18; 1982.
13. Brady, K. T.; Lydiard, R. B.; Ballenger, J. C.; Shook, J.; Laraia, M.; Fossey, M.: CSF opioids in panic disorder. Biol. Psychiatry 30:512–514; 1991.
14. Broekkamp, C. L.; Rijk, H. W.; Joly-Gelouin, D.; Lloyd, K. L.: Major tranquilizers can be distinguished from minor tranquilizers on the base of effects on marble burying and swim-induced grooming in mice. Eur. J. Pharmacol. 126:223–229; 1986.
15. Bruns, R. F.; Katims, J. J.; Annau, Z.; Snyder, S. H.; Daly, J. W.: Adenosine receptor interactions and anxiolytics. Neuropharmacology 22:1523–1529; 1983.
16. Burov, I. V.; Borisenko, S. A.: The effect of neurotropic substances on the self-stimulation reaction at the thalamic level (in Russian). Biull. Eksp. Biol. Med. 81:43–45; 1976.
17. Carmody, J.; Jamieson, D.; de Poortere, R.: Opioid-independent hyperalgesia induced in mice be pentobarbitone at low dosage. Naunyn Schmiedebergs Arch. Pharmacol. 334:193–195; 1986.
18. Chiou, L. C.; Ling, J. Y.; Chang, C. C.: Enhancement by benzodiazepines of the inhibitory affect of adenosine on skeletal neuromuscular transmission. Br. J. Pharmacol. 116:1870–1874; 1995.
19. Cohen, G. A.; Doze, V. A.; Madison, D. V.: Opioid inhibition of GABA release from presynaptic terminals of rat hippocampal interneurons. Neuron 9:325–335; 1992.
20. Cooper, K. A.; Carmody, J.: The characteristics of the opioid-related analgesia induced by the stress of swimming in mice. Neurosci. Lett. 31:165–170; 1982.
21. Crawley, J. N.: Neuropharmacological specificity of a simple animal model for the behavioral actions of benzodiazepines. Pharmacol. Biochem. Behav. 15:359–362; 1981.
22. Delong, R. E.; Phillis, J. W.; Barraco, R. A.: A possible role of endogenous adenosine in the sedative action of meprobamate. Eur. J. Pharmacol. 118:359–362; 1985.
23. Dienstbier, R. A.; Crabbe, J.; Johnson, G. O.; Thorland, W.; Jorgensen, J. A.; Sadar, M. M.; La Velle, D. C.: Exercise and stress tolerance. In: Sacks, M. H.; Sachs, M. L., eds. Psychology of running. Champaign, IL: Human Kinetics Publishers; 1981:193–210.
24. Dingledine, R.; Iversen, L. L.; Breuker, E.: Naloxone as a GABA antagonist: Evidence from iontophoretic, receptor binding and convulsant studies. Eur. J. Pharmacol. 47:19–27; 1978.
25. Duka, T.; Cumin, R.; Haefely, W.; Herz, A.: Naloxone blocks the effect of diazepam and meprobamate on conflict behavior in rats. Pharmacol. Biochem. Behav. 15:115–117; 1981.

26. Duka, T.; Millan, M. J.; Ulsamer, B.; Doenicke, A.: Naloxone attenuates the anxiolytic action of diazepam in man. Life Sci. 31:1833–1836; 1982.
27. Fernández-Guardiola, A.; Pellicer, F.; Rocha, L.; Gutiérrez, R.: Naloxone facilitates sensory precipitation of focal and generalized seizures—Evoked potentials and and power spectral-analysis in the cat. Exp. Neurol. 101:159–175; 1988.
28. Florio, C.; Prezioso, A.; Papaioannou, A.; Vertua, R.: Adenosine A_1 receptors modulate anxiety in CD1 mice. Psychopharmacology (Berlin) 136:311–319; 1998.
29. Geller, I.; Seifter, J.: The effects of meprobamate, barbiturates, *d*-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 1:482–492; 1960.
30. Goldinger, A.; Müller, W. E.; Wollert, U.: Inhibition of glycine and GABA receptor binding by several opiate agonists and antagonists. Gen. Pharmacol. 12:477–479; 1981.
31. Griebel, G.; Belzung, C.; Misslin, R.; Vogel, E.: The free-exploratory paradigm: An effective method for measuring neophobic behavior in mice and testing potential neophobia-reducing drugs. Behav. Pharmacol. 4:637–644; 1993.
32. Haefely, W.; Schaffner, P.; Polc, P.; Pieri, L.: General pharmacology and neuropharmacology of propanediol carbamates. Handb. Exp. Pharmacol. 55:263–283; 1981.
33. Harrington, M. A.; Oksenberg, D.; Peroutka, S. J.: 5-Hydroxytryptamine$_{1A}$ receptors are linked to a G_i-adenylatecyclase complex in rat hippocampus. Eur. J. Pharmacol. 154:95–98; 1988.
34. Howard, J. L.; Rohrbach, K. W.; Pollard, G. T.: Cumulative dose–effect curves in a conflict test with incremental shock. Psychopharmacology (Berlin) 78:195–196; 1982.
35. Jain, N.; Kemp, N.; Adeyemo, O.; Buchanan, P.; Stone, T. W.: Anxiolytic activity of adenosine receptor activation in mice. Br. J. Pharmacol. 116:2127–2133; 1995.
36. Johnson, S. W.; North, R. A.: Opioids excite dopamine neurons by hyperpolarizing local interneurons. J. Neurosci. 12:483–488; 1992.
37. Jones, L. S.: Naloxone blocks antiepileptogenic properties of an in vitro electroconvulsive shock model. Brain Res. 564:336–340; 1991.
38. Koob, G. F.; Strecker, R. E.; Bloom, F. E.: Effects of naloxone on the anticonflict properties of alcohol and chlordiazepoxide. Subst. Alcohol Actions/Misuse 1:447–457; 1980.
39. Kopp, C.; Vogel, E.; Rettori, M. C.; Delagrange, P.; Guardiola-Lemaitre, B.; Misslin, R.: Effects of daylight cuycle reversal on locomotor activity on several inbred strains of mice. Physiol. Behav. 63:577–585; 1998.
40. Kõks, S.; Soosaar, A.; Võkar, V.; Volke, V.; Ustav, M.; Männistö, P. T.; Bourin, M.; Vasar, E.: Opioid antagonist naloxone potentiates anxiogenic-like action of cholecystokinin agonists in elevated plus-maze. Neuropeptides 32:235–240; 1998.
41. Kramer, K.; Voss, H. P.; Grimbergen, J.; Bast, A.: Circadian rhythms of heart rate, body temperature, and locomotor activity in freely moving mice measured with radio telemetry. Lab. Anim. 27:23–26; 1998.
42. Lenègre, A.; Avril, I.; Fromage, S.; Milinkevitch, D.; Porsolt, R. D.: Absence of amnesia induction in mice with hydroxyzine in comparison with three other minor tranquilizers. Arzneimittelforschung 38:558–560; 1988.
43. Liebman, J. M.: Anxiety, anxiolytics and brain reinforcement. Neurosci. Biobehav. Rev. 9:75–86; 1985.
44. Lupica, C. R.: δ and μ enkephalins inhibit spontaneous GABA-mediated IPSCs via a cyclic AMP-independent mechanism in the hippocampus. J. Neurosci. 15:737–739; 1995.
45. Macksay, G.; Ticku, M. K.: CNS depressants accelerate the dissociation of ^{35}S-TSBS binding and GABA enhances their displacing potencies. Life Sci. 43:1331–1337; 1988.
46. Martin, D. C.; Introna, R. P.; Aronstam, R. S.: Fentanyl and sufentanyl inhibit agonist binding to 5-HT_{1A} receptors in membranes from the rat brain. Neuropharmacology 30:323–327; 1991.
47. Metha, A. K.; Kulkarni, S. K.: Mechanisms of potentiation by diazepam of adenosine. Life Sci. 34:81–86; 1984.
48. Miczek, K. A.; Thompson, M. L.: Analgesia resulting from defeat in a social confrontation: The role of endogenous opioids in the brain. In: Bandler, R., ed. Modulation of sensorimotor activity during alterations in behavioral states. New York: Alan R. Liss; 1984:431–456.
49. Misslin, R.; Belzung, C.; Vogel, E.: Behavioural validation of a light/dark choice procedure for testing anti-anxiety agents. Behav. Proc. 18:119–132; 1989.
50. Moroi, K.; Ozaki, N.; Kadota, T.; Kadota, K.: Characterization of [H-3]5-hydroxytryptamine and [H-3]spiperone binding sites in clathrin-coated vesicles from bovine brain. Brain Res. 794:291–298; 1998.
51. Onaivi, E. S.; Martin, B. R.: Neuropharmacological and physiological validation of a computer-controlled two-compartment black and white box for the assessment of anxiety. Prog. Neuropsychopharmacol. Biol. Psychiatry 13:964–976; 1989.
52. Patel, J. B.; Martin, C.; Malick, J. B.: Differential antagonism of the anticonflict effects of typical and atypical anxiolytics. Eur. J. Pharmacol. 86:295–298; 1983.
53. Paul, S. M.; Marangos, P. J.; Skolnick, P.: The benzodiazepine–GABA–chloride ionophore receptor complex: Common site of minor tranquilizer action. Biol. Psychiatry 16:213–229; 1983.
54. Peeler, D. F.; Nowakowski, R. S.: Genetic factors and the measurement of exploratory activity. Behav. Neural Biol. 48:90–103; 1987.
55. Pellicer, F.; Rocha, L.; Gutiérrez, R.; Fernández-Guardiola, A.: Epileptogenesis and muscular hypertonic postictal phenomena induced by naloxone in intact cats. Epilepsia 29:374–378; 1988.
56. Phillis, J. W.: Adenosine's role in the central actions of benzodiazepines. Prog. Neuropsychopharmacol. Biol. Psychiatry 8:495–502; 1984.
57. Phillis, J. W.; Delong, R. E.: A purinergic component in the central actions of meprobamate. Eur. J. Pharmacol. 101:295–297; 1984.
58. Phillis, J. W.; O'regan, M. H.: The role of adenosine in the central actions of the benzodiazepines. Prog. Neuropsychopharmacol. Biol. Psychiatry 12:389–404; 1988.
59. Pollard, G. T.; Howard, J. L.: The staircase test: Some evidence of nonspecificity of anxiolytics. Psychopharmacology (Berlin) 89:14–19; 1986.
60. Rho, J. M.; Donevan, S. D.; Rogawski, M. A.: Barbiturate-like actions of propanediol dicarbamates felbamate and meprobamate. J. Pharmacol. Exp. Ther. 280:1383–1391; 1997.
61. Sanger, D. J.; Joly, D.; Zivkovic, B.: Behavioral effects of non-benzodiazepine anxiolytic drugs: A comparison of CGS 9896 and zopiclone with chlordiazepoxide. J. Pharmacol. Exp. Ther. 232:831–837; 1985.
62. Sarne, Y.; Fields, A.; Keren, O.; Gafni, M.: Stimulatory effects of opioid transmitter release and possible cellular mechanisms: Overview and original results. Neurochem. Res. 21:1353–1361; 1996.
63. Shanks, N.; Griffiths, J.; Zalcman, S.; Zacharko, R. M.; Anisman, H.: Mouse strain differences in plasma corticosterone following uncontrollable footshock. Pharmacol. Biochem. Behav. 36:515–519; 1990.
64. Simiand, J.; Keane, P. E.; Morre, M.: The staircase test in mice: A simple and efficient procedure for primary screening of anxiolytic agents. Psychopharmacology (Berlin) 84:48–53; 1984.
65. Soubrié, P.; de Angelis, L.; Simon, P.; Boissier, J. R.: Effets des anxiolytiques sur la prise de boisson en situation nouvelle et familière. Psychopharmacologia 50:41–45; 1976.
66. Soubrié, P.; Jorbert, A.; Thiebot, M. H.: Differential effects of naloxone against the diazepam-induced release of behavior in rats in three aversive situations. Psychopharmacology (Berlin) 69:101–105; 1980.
67. Soubrié, P.; Kulkarni, S.; Simon, P.; Boissier, J. R.: Effets des anxiolytiques sur la prise de nourriture de rats et de souris placés en situation nouvelle et familière. Psychopharmacologia 45:203–210; 1975.
68. Spyraki, C.; Kazandjian, A.; Varonos, D.: Diazepam-induced place preference conditioning: Appetitive and antiaversive properties. Psychopharmacology (Berlin) 87:225–232; 1985.
69. Sudak, H. S.; Maas, J. W.: Central nervous serotonin and norepinephrine localization in emotional and non-emotional strains of mice. Nature 203:1254–1256; 1964.

70. Thompson, W. R.: The inheritance of behavior: Behavioral differences in fifteen mouse strains. Can. J. Psychol. 7:145–155; 1953.
71. Trillat, A. C.; Malagie, I.; Mathe-Allainmat, M.; Anmela, M. C.; Jacquot, C.; Langlois, M.; Gardier, A. M.: Effects of WAY 100635 and (−)-5-Me-(-OH-DPAT, a novel 5-HT_{1A} receptor antagonist, on 8-OH-DPAT responses. Eur. J. Pharmacol. 347:41–49; 1996.
72. Trullas, R.; Skolnick, P.: Differences in fear motivated behaviors among inbred mouse strains. Psychopharmacology (Berlin) 111:323–331; 1993.
73. Tsuda, M.; Suzuki, T.; Misawa, M.; Nagase, H.: Involvement of the opioid system in the anxiolytic effect of diazepam in mice. Eur. J. Pharmacol. 307:7–14; 1996.
74. Vogel, J. R.; Beer, B.; Clody, D. E.: A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacologia 21:1–7; 1971.
75. Zaleska-Freylan, K. I.: Diurnal changes in the level of blood plasma corticosteroids in laboratory mice in relation to sex and season. Bull. Acad. Polonaise Sci. Series Sci. Biol. 27:571–574; 1979.
76. Zangrossi, H.; Leite, J. R.; Graeff, F. G.: Anxiolytic effect of carbamezapine in the elevated plus-maze: Possible role of adenosine. Psychopharmacology (Berlin) 106:85–89; 1992.