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GABA Receptor-Linked Chloride Channels and the Behavioral Effects of Naltrexone in Rats

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GEWISS, M. V., R. J. MARLEY, E. B. THORNDIKE, S. R. GOLDBERG AND C. W. SCHINDLER. *GABA receptor-linked chloride channels and the behavioral effects of naltrexone in rats.* PHARMACOL BIOCHEM BEHAV **49**(3) 589–597, 1994.—The present study was conducted to determine whether the effects of naltrexone on schedule-controlled behavior in rats were mediated, at least in part, by the GABAergic system. Because the enhanced sensitivity that has been shown to occur following naltrexone treatment might alter the effects of the treatment compounds, a variety of compounds interacting with the GABA system were tested in both sensitized and nonsensitized animals. Of all the compounds tested in this manner, only the dose-effect function for the GABAA agonist muscimol was altered by the naltrexone treatment, with the higher doses of muscimol producing response-rate decreasing effects only in naltrexone-sensitized rats. In the naltrexone-treated animals, these same GABA agonists and antagonists were used as pretreatments prior to the determination of the naltrexone dose–effect function. Although shifts in the naltrexone dose–effect function were observed, the effects were not consistent either within or across receptor class. In contrast, the chloride-channel antagonist picrotoxin clearly shifted the naltrexone dose–effect function in sensitized animals to the left, while the chloride-channel facilitator pentobarbital shifted the function to the right. These results indicate that the effects of naltrexone are at least partially mediated by an action at the GABA-linked chloride channel, rather than directly at the GABA receptor.

Naltrexone GABA agonists GABA antagonists Pentobarbital Picrotoxin
Schedule-controlled behavior Rats

IN ADDITION to interactions with opioid receptors, evidence indicates that many opioid antagonists may also have effects on gamma-aminobutyric acid (GABA) receptor (14,20). At high doses (3), these opioid antagonists act as GABA antagonists as measured by their effects on receptor binding (6), their ability to produce convulsions in mice and rats (19), and their ability to increase cerebellar cyclic GMP content (13). Opioid antagonists also have been shown to decrease cerebellar GABA content (17). In a drug discrimination study, Carter and Leander (4) demonstrated that picrotoxin generalized to naloxone, suggesting that the pharmacological actions of opioid antagonism and GABA antagonism may both contribute to the naloxone discriminative stimulus complex. Carter et al. (5) pointed out that chronic diazepam shifted the dose–effect curve for naloxone and picrotoxin to the right in pigeons responding under a multiple schedule of food presentation. It has also been shown that chronic administration of diazepam, which is known to facilitate GABA-mediated synaptic inhibition, produces an attenuation of the rate-decreasing effects of naloxone in naloxone-sensitive animals (8). These results suggest that the effects of naloxone or naltrexone on schedule-controlled responding may be due to an antagonism of GABA neurotransmission.

Additional biochemical evidence of an interaction between the opioid and GABA systems comes from studies of changes in GABAergic function associated with opioid antagonist enhanced sensitivity. Schindler et al. (22) have shown that following the development of enhanced sensitivity to naltrexone in rats, the ability of GABA to stimulate the uptake of chloride ions into vesicular membrane preparations was unchanged in the cortex, but increased in the cerebellum. These results suggest that the effects of naltrexone on cerebellar GABA receptors may be associated with the development of enhanced sensitivity to opioid antagonists.

Taken together, the neurochemical and behavioral evidence indicates a link between the opioid and GABA neuro-

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transmitter systems. The present study was conducted to determine whether the behavioral effects of naltrexone in naltrexone-sensitized rats could be altered by specific GABA agonists or antagonists. Because sensitization develops rapidly in rats treated with cumulative doses of naltrexone (21), the current experiments were performed in naltrexone-sensitized rats, so as to establish a stable baseline of behavior. However, because the development of sensitization may also have altered the effects of the GABAergic agents, these agents were tested alone (without naltrexone) in both sensitized and nonsensitized rats. The $GABA_A$ drugs tested were the agonists muscimol and THIP and antagonists bicuculline and SR95531. The $GABA_B$ drugs tested were the agonist baclofen and antagonists DANVA, phaclofen and 2-hydroxy-saclofen. Knowing that $GABA_A$ receptors are coupled to ionophores that permit the influx of chloride with resultant hyperpolarization of neuronal membranes (9), additional experiments were conducted with pentobarbital and picrotoxin, agents that directly modify the functioning of the chloride ion channel. Specifically, barbiturates act by enhancing the inhibitory synaptic actions of GABA (15). They can reverse picrotoxin antagonism of several GABA-mediated neuronal responses and are capable of potentiating GABA-induced increases in chloride conductance. Thus, pentobarbital appears to increase the lifetime of the open state of chloride channels that are regulated by GABAergic receptors (24). On the contrary, picrotoxin selectively antagonizes the effects of GABA by interacting with sites associated with the chloride ionophore (11).

METHOD

Subjects

Male Long-Evans rats (Harlan Sprague-Dawley, Indianapolis IN), individually housed with free access to water, were food deprived to 80% of their free feeding weight (300–400 g) and maintained at those weights throughout the experiment. Tests were conducted during normal working hours (0800–1700 h), 5 days per week.

Apparatus

The subjects were trained in four identical operant conditioning chambers (31 × 25 × 27 cm), with the front and rear walls made of aluminum and the two sides and ceiling made of transparent Plexiglas. The floor was made up of 16 stainless steel grids, 0.5 cm in diameter, and spaced 2.0 cm apart. On the front wall, 3 cm from the left wall and 4 cm above the grid floor, was a 2.0-cm diameter response key (Gerbrands model B, Arlington, MA). The key could be transilluminated with an orange light, and a horizontal force of 0.15 N was required to operate the key. Also, centered on the front wall, 1.5 cm above the grid floor, was a 4.0 × 4.5 cm opening for a food trough into which 45-mg food pellets (Bioserv #0021, Frenchtown, NJ) were delivered. A houselight was located in the corner of the ceiling and the front wall. The entire experimental chamber was enclosed in an 51 × 35 × 37 cm ventilated acoustical chamber (BRS/LVE, Laurel, MD). All experimental events and data collection were performed using a Med-PC (Med Associates) computer system.

Procedure

The procedure used to induce enhanced sensitivity to naltrexone has been described previously by Schindler et al. (21). Rats were initially trained to press the key with food reinforcement by the method of successive approximation (10). During this training, the key was lighted with the orange light and the houselight was on. Whenever a food pellet was delivered, both the keylight and houselight were turned off for 2 s. Once the rats were reliably pressing the key, the number of responses required was gradually increased over several sessions until every 30th response produced a food pellet (a fixed ratio 30 schedule of food presentation, FR 30). After responding stabilized on the FR30 schedule, a timeout was instituted prior to the session. During the timeout, no lights were on in the experimental chamber and responding had no scheduled consequence. The timeout was initially 30 s. The timeout was followed by a 3-min period during which the keylight and houselight were on, and responses were reinforced according to the FR 30 schedule. This period was followed by another timeout, a 3-min FR period and so on, until 5 FR periods had occurred. The timeouts preceding each FR period were gradually increased to 10 min. Thus, the final training schedule consisted of five 3-min periods of FR 30 reinforcement, each preceded by a 10-min timeout. Once responding had stabilized, saline was occasionally administered approximately 2-min into each 10-min timeout to adapt the rats to the injection regimen. Injections were given on Wednesdays and Fridays until it was established that these saline injections did not disrupt responding.

From this point, the animals were divided into two groups. Eight animals received five injections of saline on Wednesdays and Fridays (saline group), eight others received saline on Wednesdays and five increasing dosages of naltrexone (1.0, 2.0, 7.0, 20.0, and 70.0 mg/kg) on Fridays during the five successive timeout periods. Thus, the final cumulative dose for naltrexone for this group was 100.0 mg/kg. This procedure lasted for 8 weeks, during which sensitivity to naltrexone progressively developed.

Following this training, various $GABA_A$ and $GABA_B$ agonist (THIP, muscimol, baclofen) and antagonist (bicuculline, SR95531, DANVA, phaclofen, 2-hydroxy-saclofen) compounds were administered as cumulative doses to both groups. The chloride channel facilitator pentobarbital and chloride channel blocker picrotoxin were also administered to both groups. For all drugs tested, cumulative doses were injected to obtain an initial dose-response curve (cumulative dosages experiments) in both the naltrexone-sensitized and saline-treated groups. These initial doses were chosen on the basis of preliminary data and literature searches to be free of convulsant properties. Subsequently, the maximal dose without significant behavioral effects was used as pretreatment before cumulative injections of naltrexone (1, 2, 7, 20, and 70 mg/kg) for the naltrexone group. The order of testing was nonsystematic other than that the initial dose-effect functions for the individual agents were determined prior to pretreatment testing. The pretreatments were given to determine how they would affect the naltrexone dose-effects curve in sensitized animals. Periodic tests of saline alone and naltrexone alone were continued during this period of testing. Table 1 gives an outline of the experimental conditions each groups was exposed to.

Drugs

All drugs were administered intraperitoneally. Naltrexone hydrochloride, pentobarbital, picrotoxin, and gabazine (SR95531) were dissolved in sterile water. Muscimol, baclofen, phaclofen, gaboxadol (THIP), *d*-amino-n-valeric acid (DANVA) and 2-hydroxy-saclofen were dissolved in 0.9% NaCl solution (saline). Bicuculline methbromide was sus-

TABLE 1
EXPERIMENTAL CONDITIONS

	Naltrexone Group	Saline Group
1.	Behavioral training	Behavioral training
2.	8 weeks naltrexone	8 weeks saline
3.	Dose-effect determinations for all agents	Dose-effect determinations for all agents
4.	Pretreatment determinations for all agents	

pended in saline, heated to about 70°C, and then dissolved by the addition of 0.1 N HCl (10% of volume). Concentrations were adjusted so that each drug was delivered in a volume of 1 ml/kg body weight. The drugs were obtained from the following sources: naltrexone hydrochloride (Research Biochemicals Inc., RBI, Natick, MA), DANVA (Sigma Chemical Co., St. Louis, MO), (±) baclofen (RBI), (−)-bicuculline methbromide (RBI), 2-hydroxy-saclofen (RBI), muscimol hydrobromide (RBI), phaclofen (RBI), sodium pentobarbital (Sigma), picrotoxin (Sigma), SR95531 (RBI), THIP hydrochloride (RBI). The intervals (determined according to previous results in the literature) between drug injection and behavioral observation for the pretreatment experiments were the following: pentobarbital, picrotoxin, SR95531 10 min; bicuculline, baclofen, DANVA and 2-hydroxy-saclofen 20 min; muscimol and THIP 30 min.

Data Analysis

Rates of responding were determined for each FR period during a session. A measure of percent of control was assessed using the mean response rate of the closest saline session. All data were analyzed by a two-way analysis of variance (ANOVA), with post hoc Fishers LSD tests to determine individual effects. Where appropriate, ED_{50}s, along with 95% confidence limits (CL), were calculated from linear regressions.

RESULTS

Figure 1 shows the development of enhanced sensitivity to the cumulative administration of naltrexone once weekly for 8 weeks. When first administered (week 1), only the cumulative dose of 100 mg/kg decreased responding, but after 8 weeks of treatment with the opiate antagonist, a cumulative dose of 10 mg/kg suppressed responding. Statistical analysis confirmed the increased sensitivity with every week after week 2 being significantly ($p < 0.05$) different from week 1. This shift to the left of the dose-effect function was taken as evidence of enhanced sensitivity and persisted throughout the course of the experiment. Throughout the course of the experiment, response rates following the saline injections were comparable to sessions where no injections were given.

The effects of the $GABA_A$ agonists muscimol and THIP alone on response rates in the naltrexone and saline-treated rats are illustrated in Fig. 2. Cumulative dosages of the $GABA_A$ agonist muscimol produced a decrease in responding in the naltrexone group at 1 mg/kg, while little effect on responding was observed for the saline group, $F1, 35 = 34.4, p < 0.00\ 1$ (Fig. 2, left hand panel). The dose-response curves for the $GABA_A$ agonist THIP (0.08–8 mg/kg) in both the naltrexone and saline groups remained relatively flat. The effects of pretreatment with these GABA agonists on the behavioral effects of naltrexone in naltrexone-sensitized animals are also illustrated in Fig. 2. Pretreatment with muscimol (1 mg/kg) given 30 min before the naltrexone injections failed to alter the naltrexone dose-effect function (Fig. 2, right hand panels). However, pretreatment with 8 mg/kg THIP significantly shifted the naltrexone dose-effect function to the right, $F4, 40 = 2.8, p < 0.05$. This shift was also reflected as an increase in the naltrexone ED_{50} to 17.1 mg/kg (CL: 9.49–31.0) from 5.1 mg/kg (CL: 4.86–5.35) under the no pretreatment condition. Similar comparisons were made for the two $GABA_A$ antagonists bicuculline and SR95531. Cumulative dosages of neither bicuculline (0.03–2 mg/kg) nor SR95531 (0.03–2 mg/kg) produced an effect on responding for either the naltrexone group or saline group (Fig. 3, left hand panels), nor did pretreatment with bicuculline (1 mg/kg) or SR95531 (1 mg/kg) alter the naltrexone dose-effect function (Fig. 3, right hand panels).

Cumulative dosages of the $GABA_B$ agonist baclofen (0.05–5 mg/kg) did not alter responding in either the naltrexone or saline group (Fig. 4, left hand panel). On the right side of this figure are shown the effects of baclofen (5 mg/kg) in combination with the cumulative dosages of naltrexone. Pretreatment with baclofen produced a significant shift in the naltrexone dose-effect function to the right, $F1, 40 = 14.3, p$

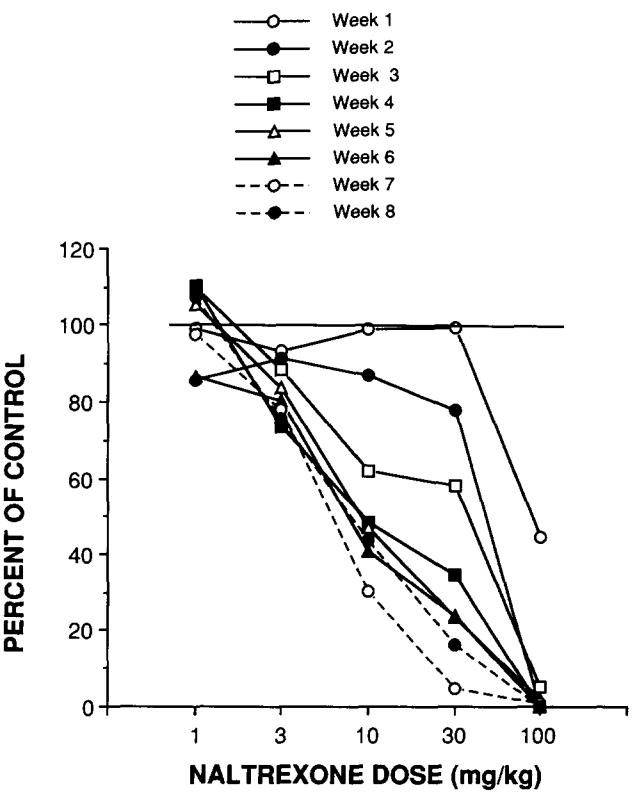

FIG. 1. Development of enhanced sensitivity to naltrexone across the first 8 weeks of the experiment. Enhanced sensitivity developed to naltrexone as evidenced by the shift in the dose-effect functions to the left. The percent of control measure was determined using the mean response rate of the two closest saline sessions as the control ($n = 8$). During the same time, the saline group displayed relatively unchanged response rates. All doses are given as the cumulative amount.

< 0.001. Again, this shift was also reflected in the naltrexone ED_{50}s, which increased to 16.59 mg/kg (CL: 10.16-27.08) following baclofen from 4.28 mg/kg (CL: 1.9-9.64) in the no pretreatment condition. Figure 5 shows that cumulative dosages of the $GABA_B$ antagonists DANVA (0.1-10 mg/kg) and phaclofen (0.1-1 0 mg/kg) produced no clear effects on responding. Although the effects of DANVA were significantly different for the naltrexone and saline groups, $F(1, 35) = 7.3$, $p < 0.01$, neither group differed greatly from control. For 2-hydroxy-saclofen (0.05-1.5 mg/kg), decreases in responding were observed above 0.15 mg/kg, but no differential effects were observed for the naltrexone and saline groups. The effects of 2-hydroxy-saclofen in doses higher than 1.5 mg/kg could not be evaluated because they induced slight convulsions in some animals. Pretreatment with DANVA (10 mg/kg) produced a shift to the right in the naltrexone dose-effect function, $F(4, 40) = 3.5$, $p < 0.05$, with the corresponding naltrexone ED_{50}s being 28.7 mg/kg (CL: 16.36-50.33) following DANVA and 9.35 (CL: 6.8-12.86) in the no pretreatment condition. Neither phaclofen (10 mg/kg) nor 2-hydroxy-saclofen (0. 15 mg/kg) had an effect as pretreatments.

Figure 6 illustrates the results with the two compounds that directly modulate the GABA-gated chloride channel. The chloride channel antagonist picrotoxin (0.008-0.56 mg/kg) had little effect on responding by itself. When given alone, pentobarbital had a similar effect in both the naltrexone and saline groups. However, pretreatment with 0.56 mg/kg picrotoxin shifted the naltrexone dose-effect function significantly to the left, $F(1, 40) = 19.7$, $p < 0.001$. In contrast, the chloride channel facilitator pentobarbital (10 mg/kg) had the opposite effect, shifting the naltrexone dose-effect function to the right, $F4, 40 = 5.1$, $p < 0.01$). For these two pretreat-

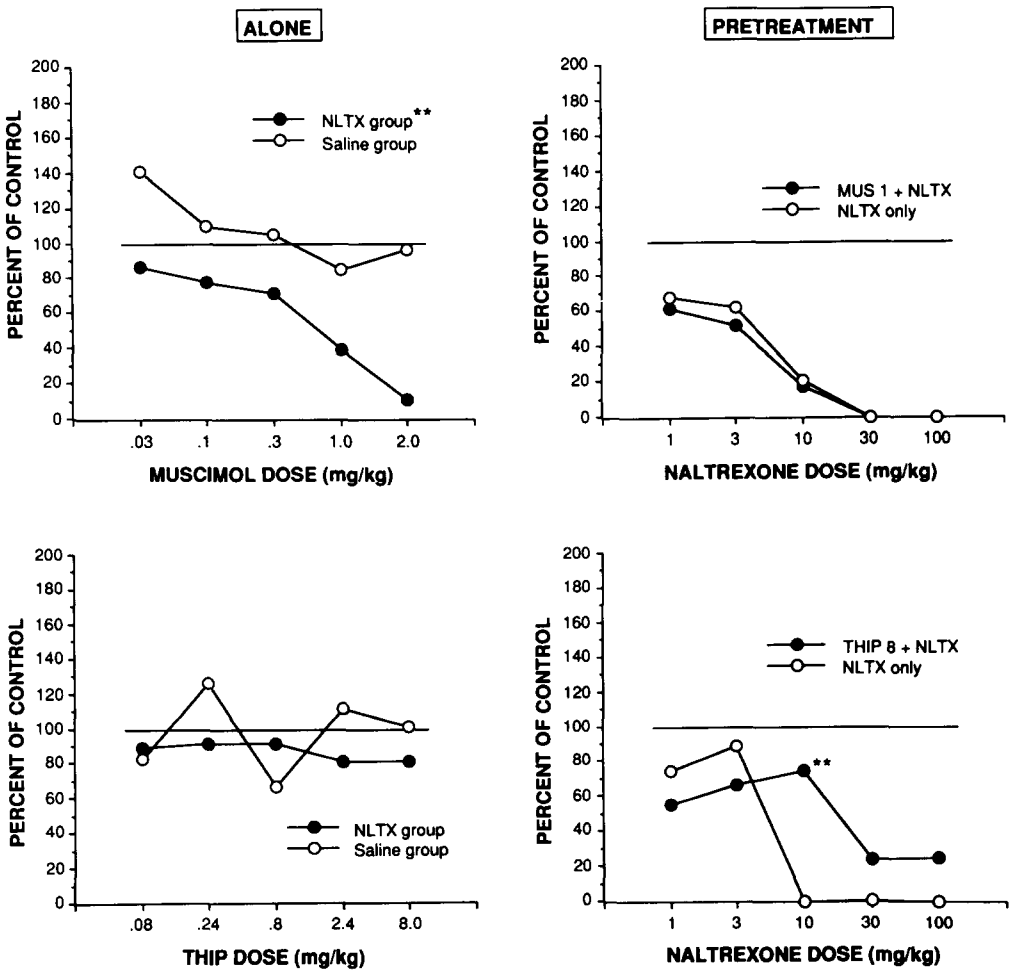

FIG. 2. The left hand panels show the effect of cumulative injections of two $GABA_A$ agonists (muscimol or THIP) on response rates in the naltrexone ($n = 5$) and saline groups ($n = 4$-5). Naltrexone was not administered during these sessions. For the right hand panels, pretreatment effects of muscimol (1 mg/kg, $n = 6$) or THIP (8 mg/kg, $n = 6$) on the naltrexone dose-effect function are compared with naltrexone administered without pretreatment (NLTX only, $n = 4$-5). The data are shown as the response rates expressed as a percentage of saline control response rates. $^{**}p < 0.01$ from saline group (left hand panels) or from the naltrexone only group (right hand panels). For this and subsequent figures, significant effects noted by figure legend indicate group differences, while those noted by individual points indicate differences only for that dose. All doses are given as the cumulative amount.

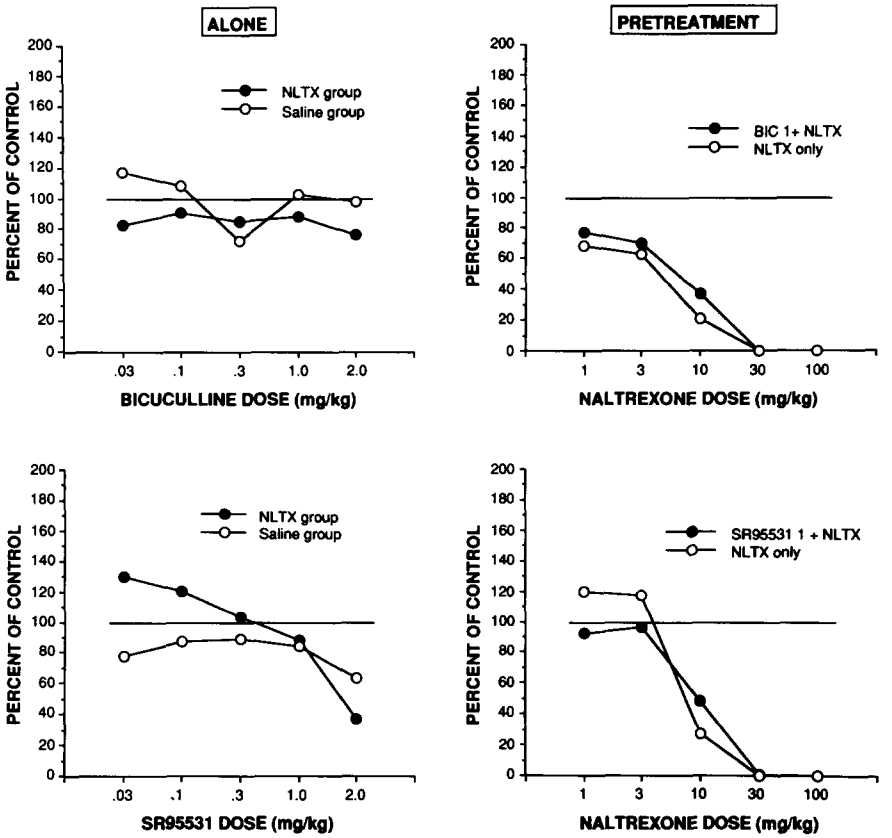

FIG. 3. The left hand panels show the effect of cumulative injections of two $GABA_A$ antagonists (bicuculline or SR95531) on response rates in the naltrexone (n = 5) and saline groups (n = 4-5). For the right hand panels, pretreatment effects of bicuculline (1 mg/kg, n = 6) or SR95531 (1 mg/kg, n = 5) on the naltrexone dose-effect function are compared with naltrexone administered without pretreatment (NLTX only, n = 5). Other details as in Fig. 2.

ment conditions, the naltrexone alone ED_{50} was 9.35 mg/kg (CL: 6.8-12.86). Picrotoxin decreased the naltrexone ED_{50} to 2.24 mg/kg (CL: 1.36-3.69) while pentobarbital increased it to 28.35 mg/kg (CL: 10.94-73.48).

DISCUSSION

The results clearly showed that repeated treatment with cumulative doses of naltrexone shifted the dose-effect function for naltrexone to the left, indicating the development of enhanced sensitivity that persisted through testing. These results support earlier findings that rats and squirrel monkeys display long-lasting enhanced sensitivity to the behavioral effects of opioid antagonists after repeated administration (12,21-23). This development of enhanced sensitivity to naltrexone most probably reflects a learned behavioral response (21). The current study was designed to investigate the potential involvement of GABAergic processes in the effects of naltrexone in these naltrexone-sensitized animals.

Of the all the GABA agonists and antagonists tested in the absence of naltrexone, only the $GABA_A$ agonist muscimol showed a significant difference in effect across the naltrexone and saline groups. A group difference was also observed for DANVA; however, for both the saline and naltrexone groups, rates of responding were near control levels at all DANVA doses tested. For many of the other compounds, response rates were unaffected by even the highest dose tested. The possibility of testing higher doses was often hampered by the fact that many of these compounds are potent convulsants. However, significant dose-effects were observed for the $GABA_A$ antagonist SR95531, the $GABA_B$ antagonist 2-hydroxy-saclofen and pentobarbital. Therefore, the shift observed for muscimol would appear to be specific. However, the fact that the dose-effect function for THIP was not altered tempers the conclusion that the effect on the muscimol dose-effect function is $GABA_A$ specific. Part of the reason for the failure to observe a shift for THIP when injected in cumulative doses may be that we were not able to reach a dose of THIP that decreased responding. Nevertheless, actions at receptors other than the $GABA_A$ receptor must also be considered. It has been shown that the antinociceptive effect of THIP is not blocked by subconvulsant doses of bicuculline, suggesting an action of THIP at non-$GABA_A$ sites (25). Moreover, a number of actions of both THIP and muscimol are not blocked by $GABA_A$ antagonists (18).

Although naltrexone treatment had minimal effects on the dose-effect functions of these compounds when administered alone, a variety of compounds shifted the sensitized naltrexone dose-effect function when administered as pretreatments. The $GABA_A$ agonists THIP, the $GABA_B$ agonist baclofen,

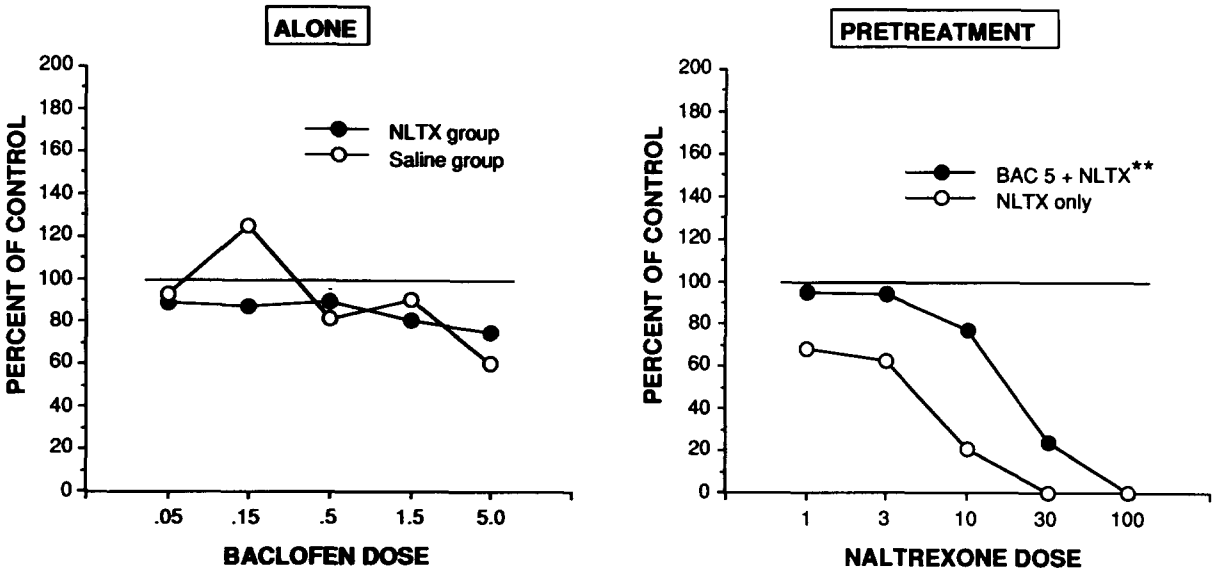

FIG. 4. The left hand panel show the effect of cumulative injections of the $GABA_B$ agonist baclofen on response rates in the naltrexone (n = 6) and saline groups (n = 5). For the right hand panels pretreatment effect of baclofen (5 mg/kg, n = 5) on the naltrexone dose-effect function is compared with naltrexone administered without pretreatment (NLTX only, n = 5). Other details as in Fig. 2.

and the $GABA_B$ antagonist DANVA all produced significant shifts to the right in the naltrexone dose-effect function for animals made sensitive to naltrexone. These results appear to suggest that naltrexone's action are mediated, at least in part, by an action at the GABA receptor. However, these effects were not consistent within class. Although DANVA produced a clear shift in the naltrexone dose-effect function, neither phaclofen nor 2-hydroxy-saclofen shifted the dose-effect function. Further, if naltrexone is acting as a GABA antagonist, we would expect GABA agonists and antagonists to have opposite effects. However, both the $GABA_B$ agonist baclofen and the $GABA_B$ antagonist DANVA produced similar shifts to the right in the dose-effect function of naltrexone. Given the variety and inconsistency of drug actions within the various classes of GABA receptor agents, the possibility that these effects are mediated at a site different from the GABA receptor itself must be considered.

Because the $GABA_A$ receptor is coupled to the chloride ionophore, an action at the chloride channel may also mediate the effects of naltrexone. For example, picrotoxin does not bind to the GABA recognition site and cannot be considered as a competitive GABA antagonist. However, by its action on the chloride channel, picrotoxin blocks GABAergic transmission. In our experiments, pretreatment with picrotoxin significantly potentiated the effects of naltrexone in the naltrexone-treated group. This effect was not due to a simple additive effect of these drugs as picrotoxin alone did not affect responding in either group. Consistent with this observation is the finding that picrotoxin or the combination of low doses of naloxone and picrotoxin can mimic the discriminative effects of higher doses of naloxone in the pigeon (4).

In contrast to picrotoxin, which selectively antagonizes the effects of GABA by interacting with a site on the chloride ionophore, barbiturates appear to increase the lifetime of the open state of chloride channels that are regulated by GABAergic receptors (24). Barbiturates are known to interact with specific binding sites on the GABA-chloride channel complex and act to facilitate and prolong the chloride channel opening induced by $GABA_A$ receptor activation (2,16). Moreover, barbiturates can reverse picrotoxin antagonism of several $GABA_A$-mediated neuronal responses. It is interesting to note that the chloride channel enhancing actions of pentobarbital, and the decreased neuronal excitability produced by pentobarbital, are completely reversed by picrotoxin. This picrotoxin reversibility suggests a site of action at or near the chloride channel (7). Previous data have shown that at 18 mg/kg pentobarbital pretreatment does not modify sensitivity to naltrexone induced by acute opioid pretreatment (1). However, Carter and Leander (5) showed that pentobarbital pretreatment (3 and 10 mg/kg) would shift the naloxone dose-effect function to the right. Likewise, our results showed that pretreatment with pentobarbital shifted the naltrexone dose-effect function to the right. Again, this effect was clearly not due to a simple additive effect, as pentobarbital alone decreased responding.

Taken together, the finding that pentobarbital and picrotoxin produced clear, but opposite shifts in the naltrexone dose-effect function indicates that naltrexone's effects on food-reinforced lever pressing are mediated, at least in part by an action at the chloride ionophore. Although this effect was observed in naltrexone-sensitized animals, whether it is dependent on the development of enhanced sensitivity is unclear. With the current procedures, evidence of enhanced sensitivity can be observed following only 1-2 weeks of treatment with naltrexone (Fig. 1), making it difficult to assess the effects of the pretreatment drugs on nonsensitized naltrexone dose-effect functions. However, we were able to test the pretreatment drugs alone in naltrexone-sensitized and nonsensitized animals. If the observed enhanced sensitivity were mediated by changes in chloride channel function or GABA receptor function, one would expect to see changes in the effects of compounds acting at these sites. Given that this was not the case, and that previous investigators have shown similar interactions between naltrexone and agents that modify chloride channel function in animals not sensitive to opioid antagonists

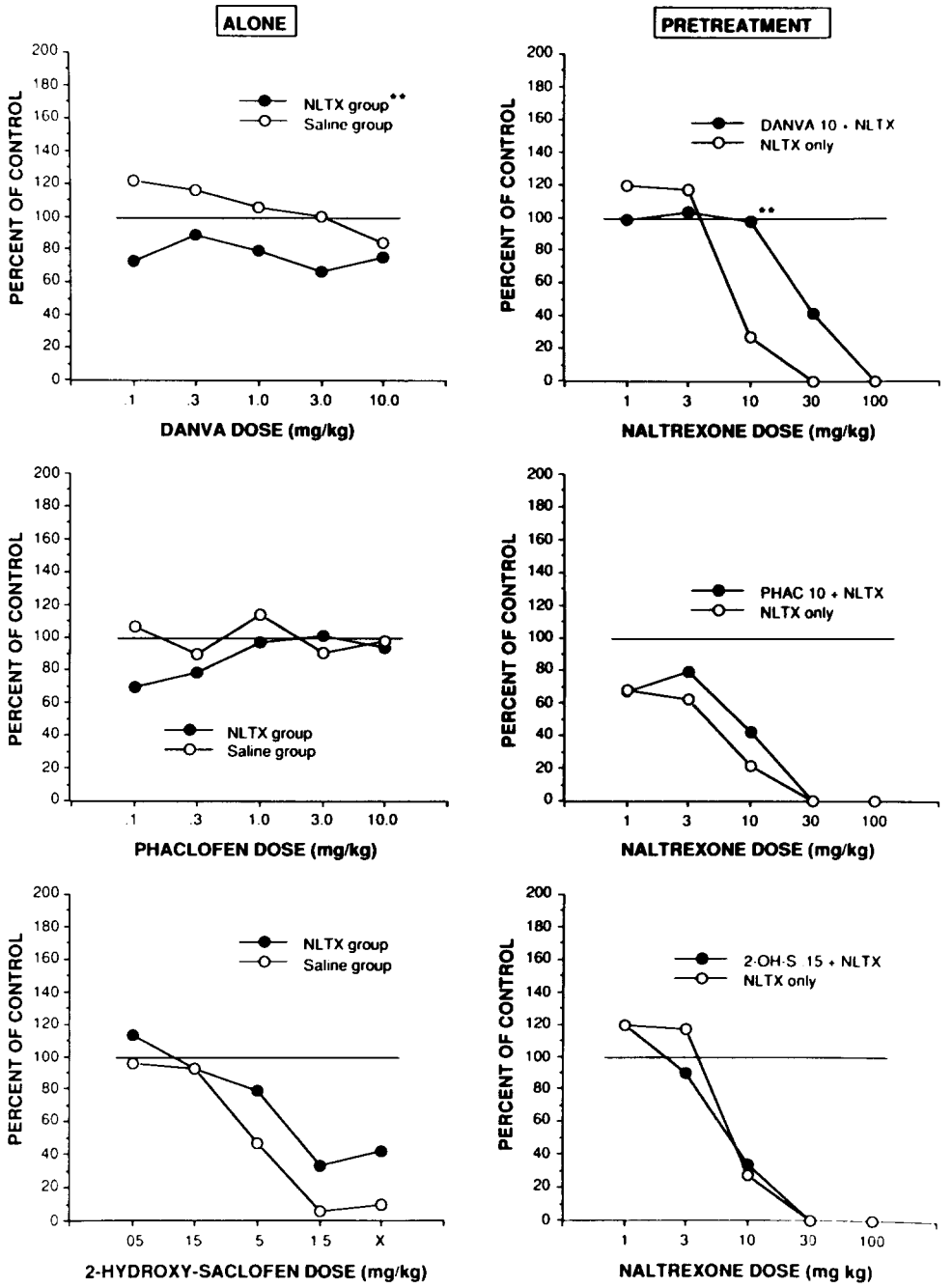

FIG. 5. The left hand panels show the effect of cumulative injections of three $GABA_B$ antagonists (DANVA, phaclofen, 2-hydroxy-saclofen) on response rates in the naltrexone (n = 4–6) and saline groups (n = 4–5). Due to slight convulsions, just four dosages were tested for 2-hydroxy-saclofen (X = absence of the 5th injection). For the right-hand panels, pretreatment effects of DANVA (10 mg/kg, n = 5), phaclofen (10 mg/kg, n = 5) or 2-hydroxy-saclofen (0.15 mg/kg, n = 5) on the naltrexone dose–effect function are compared with naltrexone administered without pretreatment (NLTX only, n = 5). Other details as in Fig. 2.

[e.g., (4)], these results suggest that the observed interactions are not specific to naltrexone-sensitized animals. However, we cannot rule out that possibility. The fact that the action of the $GABA_A$ agonist muscimol was altered by the development of enhanced sensitivity to naltrexone does indicate that an action at $GABA_A$ receptors may be related to the development of enhanced sensitivity. These results are consistent with previous finding of alterations in GABA-mediated chloride channel function in sensitized animals (22).

In summary, at least some of the behavioral effects of

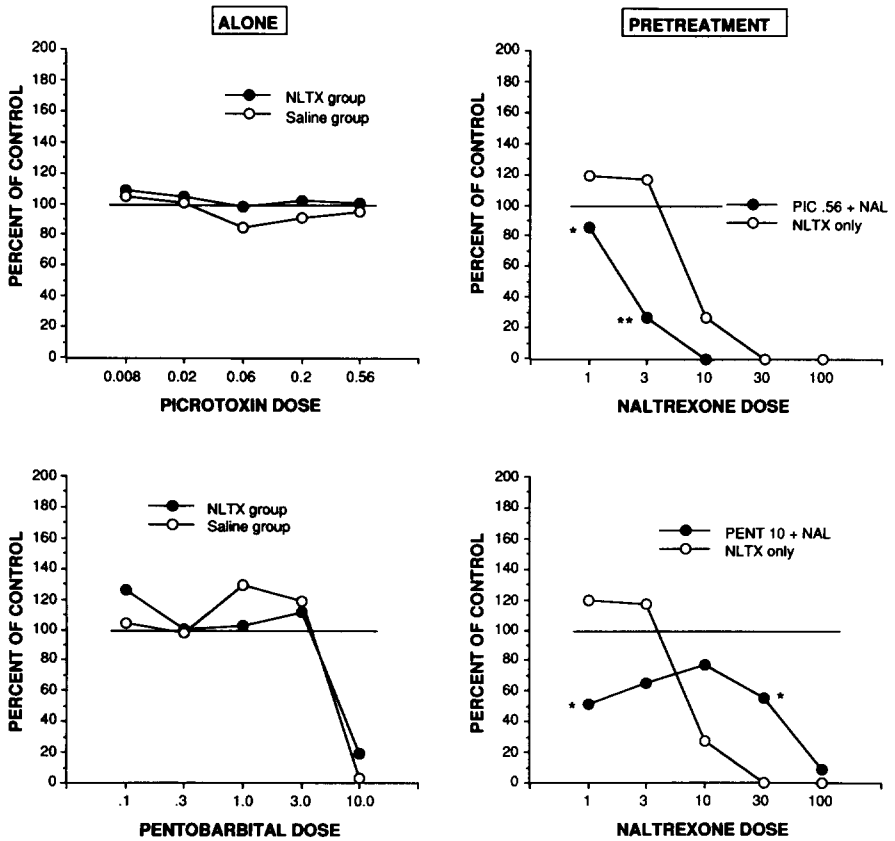

FIG. 6. The left-hand panels show the effect of cumulative injections of picrotoxin or pentobarbital on response rates in the naltrexone (n = 5) and saline groups (n = 5). For the right-hand panels, pretreatment effects of picrotoxin (0.56 mg/kg, n = 5) or pentobarbital (10 mg/kg, n = 5) on the naltrexone dose-effect function are compared with naltrexone administered without pretreatment (NLTX only, n = 5). *$p < 0.05$ Other details as in Fig. 2.

naltrexone observed in naltrexone-sensitized animals are influenced by drugs known to act on GABA-mediated synaptic transmission. The fact that the behavioral effects of muscimol were modified by the development of enhanced sensitivity to naltrexone further suggests the involvement of $GABA_A$ receptor mechanisms in the development of enhanced sensitivity to naltrexone. However, among the drugs tested as pretreatments the clearest effects were observed for those agents that directly interact with chloride channel conductance. This result indicates that, in contrast to previous suggestions, naltrexone's behavioral effects are at least partially mediated through an action at the chloride channel rather than directly at the GABA receptor. Whether this effect is the result of naltrexone acting directly at the chloride channel or indirectly through a receptor-effector system other than the $GABA_A$ receptor is unclear.

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A preliminary report appeared in Soc. Neurosci. Abst. 18:362; 1992. Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Addiction Research Center, National Institute on Drug Abuse, NIH, and the Guide for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare, Publication (NIH) 85-23, revised 1985.

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