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Tolerance to Competitive NMDA Antagonists, But No Crosstolerance With Barbiturates

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RABBANI, M., E. J. WRIGHT AND H. J. LITTLE. *Tolerance to competitive NMDA antagonists, but no crosstolerance with barbiturates.* PHARMACOL BIOCHEM BEHAV **50**(1) 9–15, 1995.—Tolerance occurred to the sedative actions of the competitive NMDA antagonists, CGP39551 and CGP37849, as measured by a decrease in spontaneous locomotor activity after 1 week or 2 weeks of administration, respectively, in studies using the TO strain of mice. Crosstolerance was seen between these compounds. When CGP37849 was given after 2 weeks treatment with CGP39551, an increase in locomotor activity was seen. Chronic barbiturate treatment, producing tolerance to the actions of pentobarbitone, did not affect the sedative properties of CGP39551 or CGP37849. Chronic treatment with CGP39551 did not alter the ataxic actions of pentobarbitone. Seven days of treatment with HA966 caused complete tolerance to its sedative actions, but no crosstolerance was seen to pentobarbitone, CGP39551, or CGP37849. A small but significant decrease was seen in the convulsion thresholds to NMDA after 15 days of treatment with CGP39551, and a small significant increase in ratings of convulsive behavior after 16 days injections of CGP37849. No significant changes were found in either B_{max} or K_d for [^{3}H]-MK-801 binding in cerebrocortical tissue 24 h after the last chronic treatment with either of the NMDA antagonists.

Tolerance NMDA antagonists Crosstolerance Barbiturates Locomotor activity CGP39551 CGP37849 TO mice

THE PHARMACOLOGICAL actions of barbiturates have long been thought to involve potentiation of the actions of the inhibitory transmitter GABA, but these compounds are now known to have actions at excitatory amino acid receptors. The central receptors for excitatory amino acids have been divided into subtypes, named after the selective agonists, the NMDA subtype, the AMPA subtype (previously known as the quisqualate subtype), and the kainate subtype (21). Noncompetitive antagonists, such as MK-801 and the general anaesthetic, ketamine, bind at a different site than the competitive antagonists, such as APV or the compounds used in the present study, CGP39551 and CGP37849 (3). Antagonist effects of barbiturates at excitatory amino acid receptors have been demonstrated, and these appear to be selective for the AMPA or kainate receptor subtypes (17,20,23).

We found recently that acute administration of the competitive antagonists, CGP39551 and CGP37849, prevented the barbiturate withdrawal syndrome in mice (14). This syndrome resembles the ethanol withdrawal syndrome and consists of tremor, hyperactivity, and convulsions. The effective doses of the competitive NMDA antagonist were lower than those required for the prevention of seizures due to NMDA in naive mice. In addition, the density of NMDA receptor-complexes, measured by the binding of [^{3}H]-MK-801, was significantly increased in cerebrocortical tissue after the chronic barbitone treatment schedule that produced tolerance and physical dependence (14). These results suggested that changes at the NMDA receptor complex may be involved in physical dependence on barbiturates.

The competitive NMDA antagonists used in these studies are from a new series of compounds, which, unlike earlier compounds, are able to pass the blood-brain barrier (3,18). These compounds, CGP37849, and its ethyl ester, CGP39551, have been demonstrated to have anticonvulsant actions when given orally. (±)-HA966 has weak partial agonist actions at the glycine site on the NMDA complex, and decreases the coagonist effects of glycine (4,5). This compound has sedative, muscle relaxant, and anticonvulsant properties (2).

The present article examines the possibility of further interactions between barbiturates and NMDA antagonists in the development of tolerance. Tolerance was measured after chronic treatment with the NMDA antagonists, and with HA966, and the presence or absence of crosstolerance with a barbiturate was determined. The occurrence of crosstolerance

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would indicate that there might be some common factor in the changes responsible for tolerance. The effects of withdrawal from the chronic treatment with NMDA antagonists were also studied, to see if any withdrawal hyperexcitability occurred. For comparison with previous work on the effects of the barbitone treatment, [^{3}H]-MK-801 binding was measured 24 h after the last of the chronic treatment injections of CGP39551 or CGP37849.

METHOD

Male mice, TO strain, between 20–35 g, with not more than 5 g range in any one experiment, were used throughout. Mice were used in one experiment only.

Chronic Treatment With CGP39551 and CGP37849

CGP39551 was given, IP, once daily, at 20 mg/kg, for 7, 9, 14, or 15 days. CGP37849 was given at 10 mg/kg, for 7 days in the initial studies, then in later experiments for 14 or 16 days, once daily. Control animals received concurrently administered saline injections. The last injections were always administered 24 h before the tests of tolerance. The doses of CGP39551 and CGP37849 were based on the doses that we found to be effective in protecting against the ethanol withdrawal syndrome (14).

Chronic Barbiturate Treatment

Barbitone was used for the chronic barbiturate treatment, because this compound is little metabolized and so does not induce microsomal enzymes. Its use, therefore, avoided the complications of metabolic tolerance. Mice were given barbitone in powdered food for 7 days: 3 mg barbitone per g food for 2 days, 4 mg/g food for 2 days, and 5 mg/g food for 3 days. Controls received a matched amount of powdered food only. All mice were weighed regularly during the treatments and no significant differences in weights were found. In all studies the amount of food and, hence, barbitone, taken in by the mice was measured daily. The barbitone intake rose during the treatment from 400 mg/kg/24 h on the first day to 700 mg/kg/24 h on the last day of treatment. The effects of CGP39551 and CGP37849 were tested 24 h after withdrawal from barbitone treatment, a time at which we have previously demonstrated tolerance to the actions of barbitone (13).

Chronic Treatment With HA966

(±)-HA966 was given by IP injection, at 5 mg/kg, once daily for 7 days. Control animals received concurrently administered saline injections. The last injections were administered 24 h before the tests of tolerance.

Locomotor Activity

The actions of the compounds on spontaneous locomotor activity were measured automatically by breaking of infrared beams. The units for the activity counts were arbitrary and based on the beam breaks by movement of mice. Pairs of mice (both from the same pretreatment) were injected with the challenge drug and then, with or without an interval, they were placed in a novel cage in the infrared apparatus. The locomotor activity was measured at 5-min interval for the next 30 min. Six pairs of mice were used for each treatment group. The treatments were randomized throughout the day, between 0900 and 1700 h, to control for diurnal variations in activity.

Ataxia

The ataxic actions of the compounds, in the tolerance studies, were measured by the rotarod method. Mice were placed on a rod rotating at 4.5 r.p.m., and the time they remained on the rod was measured automatically, at intervals after the acute administration of the drug under test. A cutoff time of 180 s was used in all experiments. Six mice were used in each treatment group.

In the studies on crosstolerance between CGP39551 and barbiturates, pentobarbitone was used as the test drug, because the ataxic effects of this compound are more clear cut than those of barbitone, which we have found to be slow in onset, with variable time of maximal effect (unpublished results). A dose of 25 mg/kg pentobarbitone was given, 24 h after the last injections of CGP39551.

Seizure Threshold Measurements

The actions of NMDA were measured by determination of the seizure thresholds by intravenous infusion (19), 24 h after the last injections of CGP39551 and 9 h after the last injections of CGP37849. NMDA, dissolved in saline at 50 mg/ml, was infused into the tail vein at 1.6 ml/min until the second of two endpoints were reached. The endpoints were the first muscle twitch, that usually preceded clonic movements, and the first signs of a full convulsion. The response times were measured by an observer who was unaware of the prior drug treatment, and the animals were humanely killed as soon as the second endpoint was reached. The thresholds were then calculated in mg/kg from the response times and the mouse weights. Between 10 and 13 mice were used for each treatment group.

Ratings of Withdrawal Hyperexcitability

Withdrawal hyperexcitability was measured by ratings of convulsive behavior on handling, a method first established by Goldstein and Pal (6) for ethanol withdrawal. Our method, that we have used for measurement of the severity of the withdrawal syndromes caused by ethanol and by barbiturates, was similar to this, with slight modifications (7). Mice were gently picked up by the tail and turned, first in one direction then the other. Rating numbers were allocated as follows: 0—no signs of tremor or hyperexcitability; 1—occasional signs of tremor; 2—continuous tremor; 3—intermittent clonic convulsions, consisting of repeated contractions of the limbs, particularly the hind legs; 4—continuous clonic convulsions. The ratings of convulsant behavior on handling were carried out once an hour, over periods of time after the last injections of CGP39551 or CGP37849. All the mice were coded so that the observer was unaware of the prior drug treatment. Ten animals were used in each treatment group.

Drugs Used

CGP39551 (DL-(E)-2-amino-4-methyl-5-phosphonopentanoate carboxyethylester, Ciba-Geigy), CGP37849 (±DL-(E)-2-amino-4-methyl-5-phosphonopentanoic acid, Ciba-Geigy), and HA966 (Tocris, 1-hydroxy-2-aminopyrrolidone-2) were dissolved in distilled water. Fresh solutions of CGP39551 and CGP37849 were made daily. *N*-methyl-D,L-aspartate (Sigma) was dissolved in distilled water. Pentobarbitone (Sigma) was

suspended in Tween 80, 0.5% v/v. The doses used of the NMDA antagonists were based on our previous work, being those effective against the barbiturate withdrawal syndrome (13).

[^{3}H]-MK-801 Binding

For the binding studies, CGP39551 was given, IP, once daily, at 20 mg/kg, for 7 days and CGP37849 at 10 mg/kg for 14 days. Control animals received concurrently administered saline injections. Dissections were carried out 24 h after the last chronic treatment injections. The animals were killed by cervical dislocation and the cerebrocortical tissues rapidly dissected out and frozen at −25°C, for a maximum of 4 weeks. The cortices from two mice were pooled for each experimental determination.

On the day of binding measurement, the frozen tissues were thawed, weighed, and suspended in assay buffer (5 mM Tris HCl, pH 7.4 at 25°C) at 55 ml per g of wet tissue, at 0–4°C. They were then hand homogenized, and the homogenate centrifuged at 1,000 × *g* for 10 min. The pellet was discarded and the supernatant was centrifuged at 20,000 × *g* for 20 min. The supernatant was discarded and the pellet was resuspended in the original volume. The suspension was finally centrifuged at 20,000 × *g* for 20 min. The resultant pellet was resuspended in 5 mM Tris HCl to give 0.5 mg/ml tissue, and kept on ice. The radioligand [^{3}H]-MK-801 (1.0 mCi/ml) [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cycloheptane-5,10-imine maleate], was diluted in glass containers with 5 mM Tris HCl, to 1.0 to 15.0 nM. To 50 μl samples from each of the diluted radioactive solutions, 4 ml of scintillation fluid was added and the mixture immediately counted on a scintillation counter. Triplicate determinations were made for each concentration. The nonspecific binding was assessed using 10 μM cold thienylcyclohexylpoperidine (TCP). Total binding was measured using 1 ml total volume of samples containing:

1. 50 μl assay buffer, 5 mM Tris HCl;
2. 100 μl assay buffer, 5 mM Tris HCl;
3. 100 μl above concentrations of [^{3}H]-MK-801;
4. 750 μl tissue aliquot.

Nonspecific binding was measured using the following:

1. 50 μl assay buffer, 5 mM Tris HCl;
2. 100 μl TCP at final concentration of 10 μM;
3. 100 μl above concentrations of [^{3}H]-MK-801;
4. 750 μl tissue aliquot.

The tubes were gently mixed and placed in a water bath at 25°C for 45 min. Following the incubation of the samples, the tubes were rapidly filtered using 30-place Brandel cell harvester and Whatman GF/B filters. A total of two washes were applied during each filtration, using ice cold 5 mM Tris HCl. Individual filter papers were removed and placed in vials, then 4 ml of scintillation fluid was added to each vial and counted on the scintillation counter, for 5 min each. To measure the amount of radioactivity put into each test tube, 50 μl of the stock and diluted [^{3}H]-MK-801 was added into a scintillation vial and counted as above.

Statistical Analysis

Comparisons in the locomotor activity studies, the measurements of seizure thresholds and latencies, and the receptor binding were made using Student's unpaired *t*-test. The rotorod results were compared using the Mann–Whitney *U*-test. The ratings of convulsive behavior were compared by two-way nonparametric analysis of variance, designed for repeat measurements (8).

RESULTS

Tolerance to the Sedative Actions of CGP39551 and CGP37849

When the locomotor effects of CGP39551, 20 mg/kg, were tested 24 h after the last of 7 days injections of this compound (Fig. 1a), it no longer decreased the locomotor activity ($p < 0.05$). The acute administration of CGP39551, 20 mg/kg, after chronic saline injections, decreased the locomotor activ-

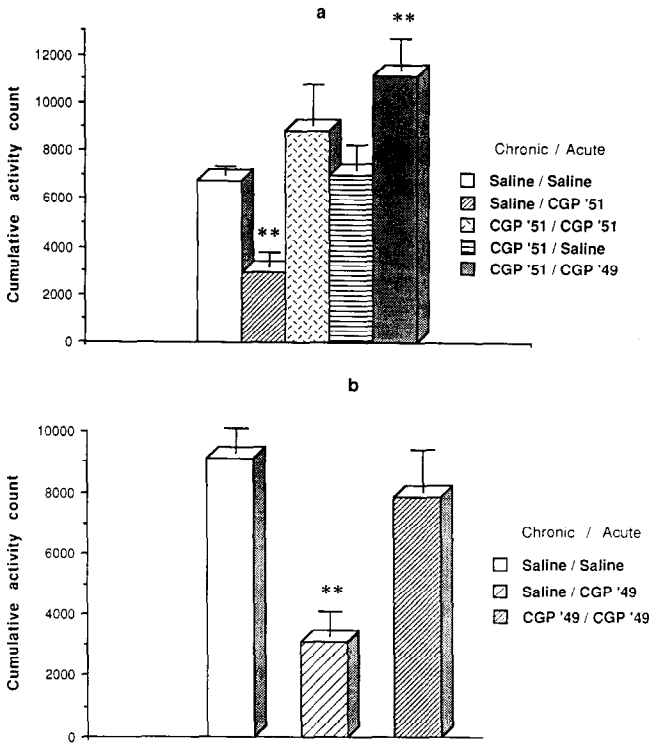

FIG. 1. (a) Tolerance to the effects of CGP39551 on spontaneous locomotor activity, and crosstolerance with CGP37849. The figure shows the locomotor counts (mean ± SE mean) over a 30-min period, beginning 1 h after acute administration of CGP39551, 20 mg/kg, CGP37849, 10 mg/kg, or saline. The tests were made 24 h after the last of 7 days injections of CGP39551. Results are expressed as mean ± SEM. A significant decrease in locomotor activity (**$p < 0.01$) was seen when CGP39551, 20 mg/kg, was given after chronic saline injections, but not when this dose was given after the chronic CGP39551 treatment (**$p < 0.001$ for comparison between CGP39551 administration after chronic saline or chronic CGP39551). When CGP37849, 10 mg/kg, was given after the chronic CGP39551, a significant increase in activity was seen (**$p < 0.01$, for comparison between the locomotor counts after chronic CGP39551 plus acute CGP37849 and chronic saline plus acute saline). (b) Illustrates the tolerance to CGP37849, measured 24 h after the last of 14 days injections of this compound. The locomotor counts (mean ± SE mean) were measured over a 30-min period, beginning 1 h after acute administration of CGP37849 or saline. The sedative action of CGP37849, 10 mg/kg, was completely prevented by the chronic treatment ($p < 0.01$ for comparison between the locomotor counts when 10 mg/kg CGP37849 was given after saline injection or after CGP37849 injections).

ity ($p < 0.001$), as we have previously shown in naive animals (13). Administration of saline 24 h after the last chronic injection of CGP39551 did not alter the activity, compared with that of mice given chronic saline injections ($p > 0.1$).

Treatment with CGP37849 for 7 days did not cause tolerance to the sedative actions of this compound (data not shown), but when it was given for 14 days, tolerance was seen. Administration of 10 mg/kg CGP37849, 24 h after the last of 14 days injections of this compound, did not cause any change in locomotor activity (Fig. 1b), while this dose gave a significant decrease in locomotor activity ($p < 0.01$) when given 24 h after the last of 14 days of saline injections.

Crosstolerance Between Compounds Acting at the NMDA Receptor Complex

When CGP37849 was given after the chronic treatment with CGP39551, the sedative actions of this compound were not only prevented, but an increase in activity was seen. When 10 mg/kg CGP37849 was given 24 h after the last of the 7 days injections of CGP39551 (Fig. 1a), the increase was significant ($p < 0.01$), compared with control values.

Tolerance to the Sedative Action of HA966

Seven daily injections of HA966 produced complete tolerance to the effects of this agent. The dose used, 5 mg/kg, caused a considerable decrease in locomotor activity after repeated saline administration, but when it was given 24 h after the last of the chronic injection of HA966, no sedation was seen, and the mean value for the locomotor activity was slightly, though not significantly, $p > 0.1$, increased (Fig. 2a).

Lack of Crosstolerance Between HA966 and CGP39551

When HA966 was given, 24 h after the last of seven daily injections of CGP39551 (Fig. 2b), it caused considerable sedation. There was no significant difference between the measurements of locomotor activity when this compound was administered after repeated saline treatment or after CGP39551 ($p > 0.1$). When CGP39551 was given 24 h after the last of the 7 days injections of HA966, it caused a significant decrease in activity; the activity did not differ from that after saline treatment (Fig. 2b). Administration of saline, 24 h after the last injections of either HA966 or CGP39551, resulted in locomotor activity levels that were not significantly different from those seen when acute saline was given after repeated saline injections, although the mean value after HA966 was higher ($p > 0.1$).

Lack of Crosstolerance With Barbiturates

When the effects of CGP39551 and CGP37849 were tested after chronic barbitone treatment, there was no evidence of crosstolerance (Fig. 3a). Our previous work has shown that our chronic barbitone treatment causes tolerance to the sedative and ataxic effects of barbitone and of pentobarbitone (13). When either CGP39551, 20 mg/kg, or CGP37849, 10 mg/kg, was given 24 h after cessation of the barbitone administration, these compounds caused significant decreases in locomotor activity (Fig. 3a). There was no significant difference between the effects of CGP37849 in mice given the control powdered food diet and those given barbitone ($p > 0.1$). After the barbitone treatment, CGP39551 caused a similar decrease in activity in barbitone-treated mice as this dose of the compound produced in naive mice in earlier studies, although in this experiment CGP39551 did not decrease the activity of animals that received the control diet ($p > 0.1$). The difference between the locomotor counts in mice withdrawn from barbitone and given acute injections of saline and controls was not significant, although the mean value after the barbitone treatment was higher than that for controls ($p > 0.05$).

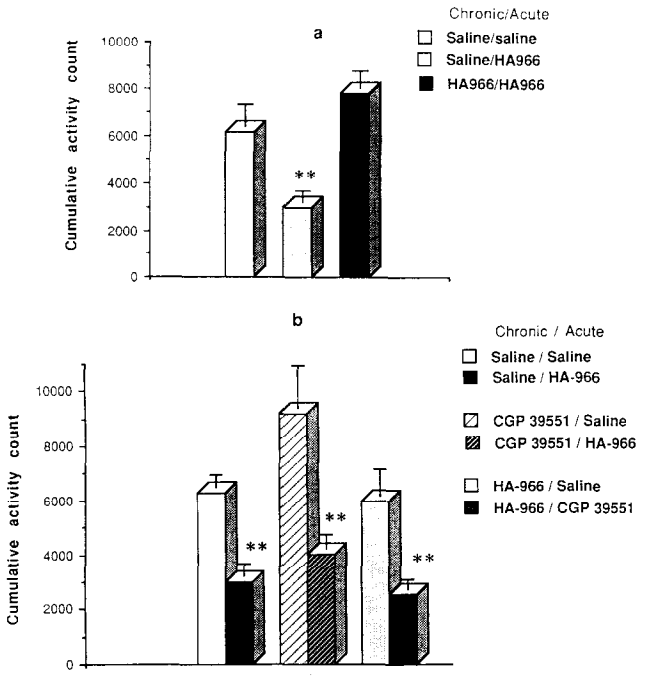

FIG. 2. (a) Shows the tolerance to the effects of HA966, 5 mg/kg, on locomotor activity when this compound was given 24 h after the last of seven daily injections with HA966, 5 mg/kg, or saline. The locomotor counts (mean ± SE mean) were measured over a 30-min period, beginning 1 h after acute administration of saline or HA966. $^{**}p < 0.01$ compared with concurrently tested controls. (b) Illustrates the lack of crosstolerance between HA966 and CGP39551. Locomotor counts (mean ± SE mean) were measured over a 30-min period, beginning 1 h after acute administration of saline, HA966, or CGP39551. The measurements were made 24 h after the last of 7 days of injections of either HA966, CGP39551 or saline. $^{**}p < 0.01$ compared with concurrently tested controls.

The ataxic actions of pentobarbitone were measured after chronic treatment with CGP39551, to see whether any crosstolerance was seen when a different test was used. The actions of pentobarbitone were unaltered by the chronic treatment (Fig. 3b). Pentobarbitone was given at 25 mg/kg, 24 h after the last of 9 days of treatment with CGP39551. There were no significant differences in the effects of pentobarbitone when compared after chronic saline or chronic drug injections ($p > 0.1$).

Effects of NMDA After Chronic Treatment With CGP39551 or CGP37849

The convulsive effects of NMDA, measured by intravenous infusion, are illustrated in Fig. 4. The thresholds for production of both muscle twitches and tonic convulsions were significantly decreased ($p < 0.01$), 24 h after the last of 15 days injections of CGP39551, 20 mg/kg (Fig. 4a). The effects of

NMDA were unaltered 9 h after the last of 15 days injections of CGP37849, 10 mg/kg (Fig. 4b).

Ratings of Convulsive Behavior on Handling After Chronic CGP39551

When ratings of convulsive behavior on handling were made between 12 h and 20 h, with additional measurements at 35 h and 38 h, after the last of 7 days repeated injections, there were no significant differences between the results from mice that received either CGP39551 or CGP37849 and those that received chronic saline injections (data not shown). When the ratings of behavior were measured from 16 h to 24 h after the last of 15 days repeated injections of CGP39551, there was no significant change in the ratings ($p > 0.1$), although the median values were slightly higher after CGP39551 during the first part of the study (Fig. 5a). However, when CGP37849 was given for 16 days, there was a small, but significant, increase in the ratings of convulsive behavior measured between 2 h and 12 h after the last of the chronic treatment injections (Fig. 5b).

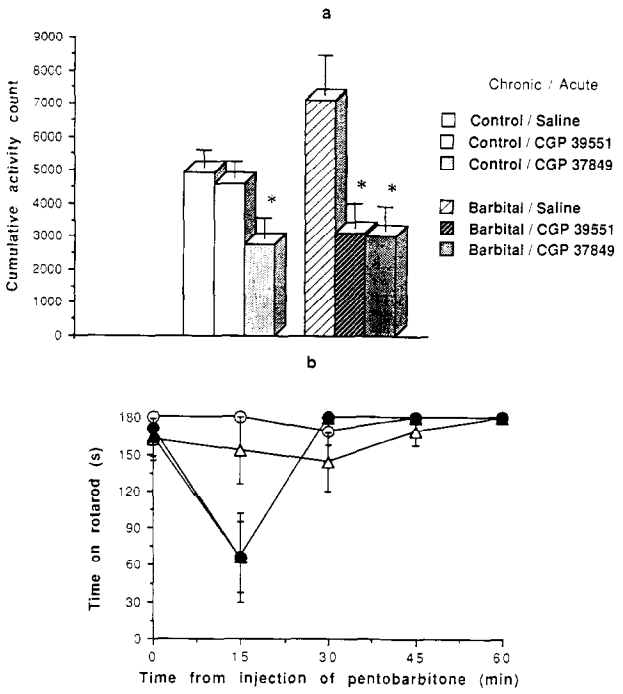

FIG. 3. (a) Shows that crosstolerance did not occur between barbitone and CGP39551 or CGP37849, when the sedative effects of CGP39551 or CGP37849, were measured, 24 h after withdrawal from 7 days of chronic barbitone treatment. Locomotor counts (mean ± SE mean) were taken over a 30-min period, beginning 1 h after acute administration of saline, CGP39551, 20 mg/kg, or CGP37849, 10 mg/kg. $^{**}p < 0.01$ compared with concurrently tested controls. (b) Lack of tolerance to the ataxic actions of pentobarbitone after chronic treatment with CGP39551. The results are given as mean ± SE mean. Acute injections of Tween vehicle or pentobarbitone, 25 mg/kg, were made at time 0, 24 h after the last of 9 days of injections of CGP39551. No significant differences were seen between the effects of pentobarbitone after chronic saline injections or after chronic treatment with CGP39551 ($p > 0.1$). ○ chronic saline injections/acute Tween vehicle; ● chronic saline injections/acute pentobarbitone; △ chronic CGP39551 injections/acute Tween vehicle; ▲ chronic CGP39551 injections/acute pentobarbitone.

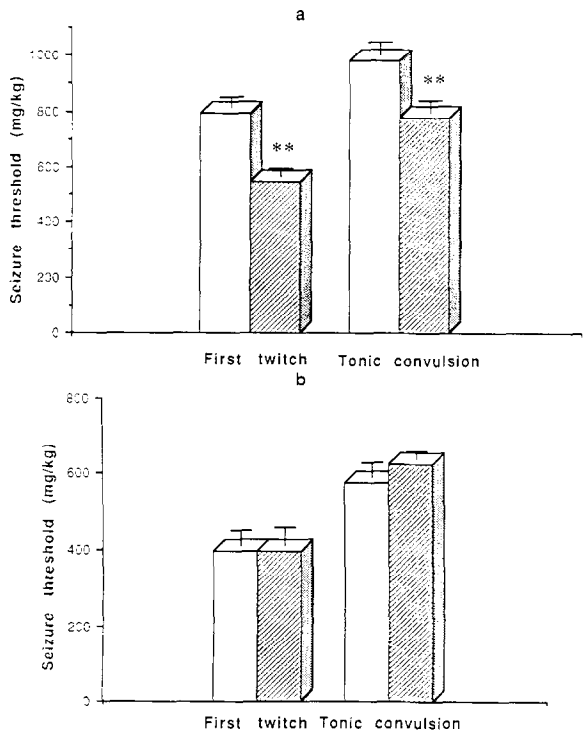

FIG. 4. (a) Seizure thresholds (mean ± SE mean) measured by intravenous infusion of NMDA, 24 h after the last of 15 days chronic treatment with CGP39551. The amounts of NMDA required to produce the first muscle twitch and to initiate a tonic convulsion were significantly decreased by the CGP39551 treatment ($^{*}p < 0.05$). Open columns are values for control animals, hatched columns represent values obtained after chronic CGP39551 treatment. (b) Seizure thresholds measured by intravenous infusion of NMDA, 9 h after the last of 15 days of chronic injections of CGP37849. The amounts of NMDA required to produce the first muscle twitch and to initiate a tonic convulsion were unaltered by the CGP37849 treatment ($p > 0.05$). Open columns are values for control animals, hatched columns represent values obtained after chronic treatment with CGP37849. Results are expressed at mean ± SE mean.

[^{3}H]-MK-801 Binding After Chronic Treatment With CGP39551 or CGP37849

The binding to [^{3}H]-MK-801 was not significantly altered by the repeated administration of CGP39551 or CGP37849. The B_{max} values are illustrated in Fig. 6a and 6c and the K_d values in Fig. 6b and 6d.

DISCUSSION

The repeated administration of the NMDA antagonists, CGP39551 or CGP37849, caused tolerance to the effects of these compounds on locomotor activity. Complete tolerance to the actions of the glycine partial agonist, HA966 on locomotor activity, was seen after only 7 days of treatment with this compound. It is possible that these results were due to changes in metabolism of CGP39551 and CGP37849, and the occurrence of metabolic tolerance cannot be ruled out until measurements of the brain concentrations of these compounds are available. However, it is also possible that changes occur at the receptor complex or the coupling with the ion channels.

There have been few studies of the effects of compounds

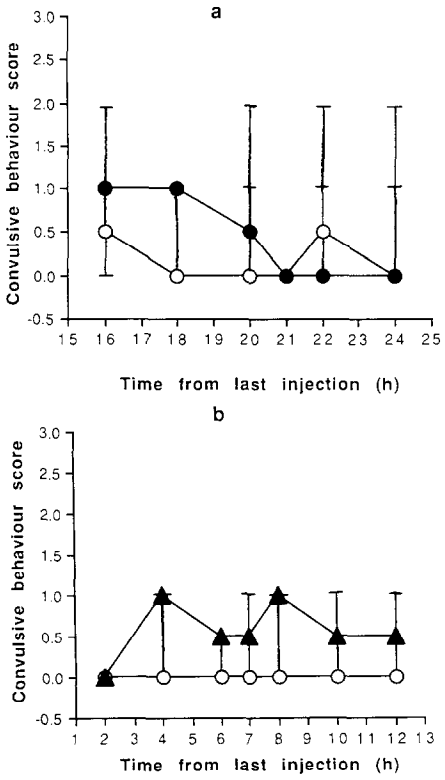

FIG. 5. (a) Ratings of convulsive behaviour on handling, expressed as median ± interquartile range, measured between 16 h and 24 h from the last of 15 days of injections of CGP39551. The differences between the ratings after saline and after CGP39551 injections were not significant ($p > 0.05$). ○ Controls; ● CGP39551. (b) Ratings of convulsive behavior on handling, expressed as median ± interquartile range, measured between 2 h and 12 h from the last of 16 days of injections of CGP37849. The ratings after chronic CGP37849 treatment were significant higher than those after the control treatment, when the values were compared over the whole of this testing time, by nonparametric analysis of variance ($p < 0.05$). ○ Controls; ● CGP37849.

acting at NMDA receptors after chronic treatment, but Boast et al. (1) demonstrated that tolerance occurred after 14 days of twice daily injections to the sedative effects of the competitive NMDA antagonist, CGS 19755, but not to the anticonvulsant action of this compound. Norman and co-workers (10) reported tolerance to the locomotor stimulating effects, and alterations in the other behavioral actions, of MK-801 after administration for 1 month.

Crosstolerance was seen between the effects of CGP39551 and CGP37849 on locomotor activity, but not between CGP39551 and HA966. After 2 weeks chronic treatment with CGP39551, not only was complete tolerance seen to the effects of CGP37849, but the effect changed from sedation to stimulation of locomotor activity. Although metabolic tolerance cannot be yet ruled out as a mechanism for the tolerance to each of these compounds, it is unlikely that increased metabolism of CGP37849 would result in stimulation of locomotor activity. An increase in locomotor activity was seen by Boast et al. (1) when the competitive NMDA antagonist, CGS19755, was given after chronic treatment with the same compound. In our studies, crosstolerance did not occur between HA966 and CGP39551, indicating that different mechanisms were involved in the development of tolerance to these compounds.

It was clear from the results that there was no crosstolerance between CGP39551 or CGP37849 and pentobarbitone, when either locomotor activity or ataxia were measured. This suggests that the mechanism of functional (cellular) tolerance to the actions of barbiturates may not involve changes at the competitive antagonist site on the NMDA receptor complex, and that different mechanisms are involved in the development of tolerance to barbiturates and to the competitive NMDA antagonists. The results also suggest that different mechanisms may be involved in barbiturate tolerance and the production of the barbiturate withdrawal syndrome, on which the NMDA antagonists had a potent protective action (14).

The repeated administration of CGP39551 and CGP37849, the competitive antagonists at the NMDA receptor, did not significantly alter [^{3}H]-MK-801 binding, even though the schedules of chronic treatment produced complete tolerance to the sedative actions of these compounds. This is in contrast to the increase in the B_{max} for [^{3}H]-MK801 binding found 24 h after withdrawal from the chronic treatment schedule for barbitone used in these tolerance studies (14,22). No change was seen in K_d after the barbitone treatment. Little study appears to have been made of receptor binding changes after chronic administration with NMDA antagonists, but down-regulation of β-adrenoceptors (11) and increased dopamine D_2 receptor synthesis (9) have been reported.

Some evidence of withdrawal changes was seen in the decreases in thresholds to NMDA after chronic CGP39551, but there appeared to be no significant withdrawal hyperexcitability when the responses to handling were measured. In the case of CGP37849, withdrawal slightly increased the ratings of convulsive behavior on handling, but the thresholds to NMDA were unchanged. The latter measurements were made

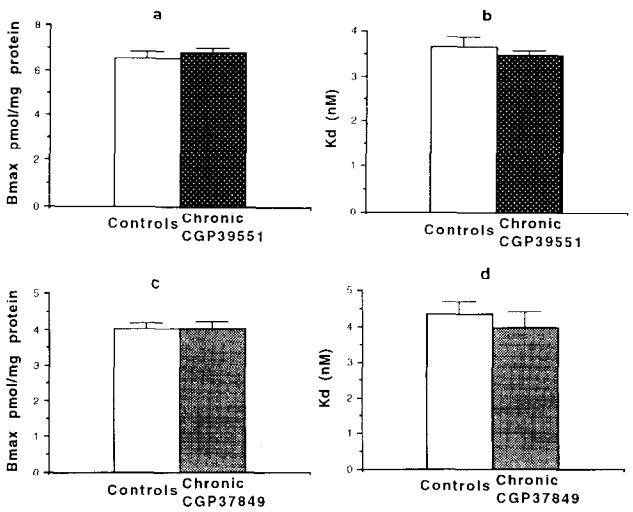

FIG. 6. Results from measurements of binding to [^{3}H]-MK-801. Parts a and c illustrate the B_{max} values and parts b and d the K_d values. The open columns are the control values and the shaded columns the values obtained after chronic treatment with either CGP39551 or CGP37849. No significant changes were produced by the repeated administration of CGP39551 or CGP37849.

at only one time point, 24 h after the last injection of CGP39551 and 9 h after the last CGP37849 injection. The duration of action of CGP39551 in our mice was more than 5 h, as the acute sedative effects were still apparent at this time, while the action of CGP37849 was shorter lasting. Withdrawal signs, if produced, would be expected to be seen when the brain concentrations were falling to their lowest levels. It appears, therefore, that these compounds produce withdrawal changes only to a very small extent after cessation of chronic treatments that were sufficient to cause complete tolerance to their sedative effects. Boast and co-workers (1) found no change in the intravenous convulsion threshold for NMDA, 20–24 h after chronic treatment with the competitive NMDA antagonist, CGP19755. With most classical sedative-hypnotic compounds, such as the barbiturates or benzodiazepines, tolerance and a withdrawal syndrome occur together as two components of physical dependence. However, although competitive antagonists at the NMDA receptor showed tolerance, withdrawal signs need not necessarily be expected, as there is evidence from studies on other compounds that these two phenomena can be separated [e.g., (15)]. On the other hand, the effects of withdrawal from benzodiazepines, for example, are not very easily seen in rodent studies, although they are now well established to occur in humans, and there remains the possibility that the same may be true of other compounds.

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