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Muscimol-Like Discriminative Stimulus Effects of GABA Agonists in Rats

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JONES, H. E. AND R. L. BALSTER. *Muscimol-like discriminative stimulus effects of GABA agonists in rats*. PHARMACOL BIOCHEM BEHAV **59**(2) 319–326, 1998.—The discriminative stimulus effects of GABAergic drugs were evaluated in rats trained to discriminate the direct $GABA_A$ agonist, muscimol (1.0 mg/kg IP), from saline under a two-lever fixed ratio (FR) 32 schedule of food reinforcement. Another direct $GABA_A$ agonist, THIP, produced full substitution for muscimol, however, at doses producing response rate decreasing effects. Diazepam, an allosteric modulator of GABA-mediated postsynaptic inhibition, yielded a maximum of 50% muscimol-lever responding at a dose that also decreased rates of responding. Partial substitution for muscimol (maximal levels of 71% muscimol-lever responding) was also produced by the GABA agonist progabide. Propofol, an anesthetic that potentiates $GABA_A$ receptor function, and the GABA uptake inhibitor, tiagabine, produced no greater than 53 and 48% muscimol-lever responding, respectively. Valproic acid, a reversible GABA transaminase inhibitor, failed to substitute for muscimol, and vigabatrin, an irreversible GABA transaminase inhibitor, yielded a maximal 46% muscimol-lever responding. These results demonstrate the pharmacological specificity of muscimol discrimination by showing that only direct agonists for the GABA site on the $GABA_A$ receptor complex produce full substitution. GABA agonists acting by other mechanisms can be distinguished from muscimol and THIP in this procedure. © 1998 Elsevier Science Inc.

Drug discrimination Muscimol GABA (gamma-aminobutyric acid) THIP Diazepam
GABA transaminase inhibitors Tiagabine Progabide Vigabatrin Valproic acid Rats

GABA (gamma-aminobutyric acid) is the major inhibitory neurotransmitter in the mammalian central nervous system (17). In addition, the $GABA_A$ subtype of GABA receptor is the site of action for a number of clinically useful drugs and chemical tools for neuropharmacology research. For example, the $GABA_A$ agonists most extensively studied are the benzodiazepines and barbiturates, which act as allosteric modulators of GABA-mediated postsynaptic inhibition. Other drugs that act on the GABA receptor complex include direct GABA agonists such as muscimol, anesthetic steroids, and possibly ethanol. In addition, GABAergic function can be indirectly altered by inhibiting GABA uptake or inhibiting its metabolism. Although all of these classes of GABA agonists can facilitate GABA-stimulated chloride flux, differences are evident in their profiles of acute pharmacological and behavioral effects (25). In this regard, drug discrimination procedures have been successfully used to compare and contrast the effect of various GABA agonists (36).

Although barbiturates, benzodiazepines, and ethanol have been extensively studied using drug discrimination procedures in animals, many of the other classes of GABA agonists have not been as well characterized. The purpose of the present study was to further examine the effects of GABAergic drugs in rats trained to discriminate the direct $GABA_A$ agonist muscimol from saline. There have been only two previous reports of using direct GABA agonists for drug discrimination training. One investigation used THIP as a training drug and reported full substitution with muscimol (1). The other study reported successfully training muscimol as a discriminative stimulus and observed substitution with THIP and antagonism by the $GABA_A$ antagonist, bicuculline (16). In that study, a representative barbiturate and benzodiazepine produced only partial substitution, pointing out the usefulness of drug discrimination procedures to distinguish among site-selective GABA agonists. The purpose of the present study was to extend this work by comparing the discriminative stimulus effects of muscimol to

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those of other types of GABA agonists. Drugs studied were the GABA pro-drug progabide, the GABA uptake inhibitor tiagabine, GABA transaminase inhibitors vigabatrin and valproic acid, and the new anesthetic propofol. Diazepam and THIP were also tested to provide reference data.

Progabide (SL 76002) is metabolized in brain to form SL 75102 [4-{[(4-chlorophenyl) (5-fluro-2-hydroxyphenyl) methylene] amino}butyric acid], a direct $GABA_A$ agonist, and then more slowly to form GABA itself (27,42). Both progabide and SL-75102 have a broad spectrum of anticonvulsant and myorelaxant effects in animal tests (42). Progabide has clinical use for the treatment of epilepsy and movement disorders (6).

Facilitation of GABAergic neurotransmission can also be achieved by inhibition of uptake of GABA into presynaptic neurons. Tiagabine prevents glial and presynaptic neuronal uptake (7) and results in increased extracellular GABA levels. In animal models (31) and clinical trials (35) tiagabine has been observed to have anticonvulsant activity.

Increases in extracellular GABA levels can also be accomplished by preventing or substantially reducing the degradation of GABA to semisuccinylaldehyde via inhibiting the enzyme GABA transaminase (30). In general, GABA transaminase inhibitors have anxiolytic, anticonvulsant, antinociceptive, and sedative effects (18,32). It has further been suggested that the inhibition of the metabolism of GABA is a fruitful mechanism for preventing seizures (13). For the present study, two GABA transaminase inhibitors were investigated, vigabatrin and valproic acid. Vigabatrin is an irreversible GABA transaminase inhibitor (22) developed for use as an anticonvulsant medication (13). Valproic acid was selected because it is a recognized inhibitor of GABA metabolism, which may exert its action through the inhibition of both GABA transaminase and semisuccinylaldehyde dehydrogenase (8,43). Valproic acid, like phenytoin and ethosuximide, also has inhibitory effects on Na^+ and Ca^+ channels that may contribute to its anticonvulsant activity (23,29).

Propofol is a new, widely used injectable anesthetic. It also has anticonvulsant effects in animal tests (20). Although the cellular mechanisms of action of propofol are still under study, it appears to act as a GABA agonist in vitro (19,37). It may have a unique mechanism for its GABAergic effects that may involve a novel binding site on the $GABA_A$-receptor complex (10,19,37). It may also act presynaptically to modify the release or reuptake of GABA (28).

METHOD

Subjects

Twelve male Sprague–Dawley rats (COBS CD) were obtained from Charles River Farms (Wilmington, MA). The animals weighed between 250 and 300 g upon arrival and were housed individually in wire mesh cages with water available ad lib. The vivarium was maintained at an ambient temperature of 22°C and a 12 L:12 D cycle. All training and testing sessions were conducted during the light phase (between 0800 and 1000 h). After completion of the training and testing sessions, rats were returned to their home cage and subsequently fed approximately 15 g of Agway rodent chow and allowed to gain weight gradually over the study for a final weight range of 360 to 420 g.

Apparatus

Six, two-lever, sound and light attenuating operant chambers (Coulbourn Instruments, Lehigh Valley, PA) were utilized in the study. Experimental sessions and data collection were executed by employing a SKED interface and SKED-11 operant conditioning software (State Systems Inc., Kalamazoo, MI) running on a DEC PDP-11BA23 minicomputer (Digital Equipment Corp., Maynard, MA). Lever pressing was reinforced with 45-mg food pellets (Dustless Precision Pellets, P. J. Noyes, Lancaster, NH) delivered into a food trough located between the two response levers. A single 8-W white houselight located approximately 20 cm above the food trough illuminated each chamber, signaling the commencement of the session.

Training Procedure

Rats were shaped to press levers for food reinforcement during daily (Monday–Friday) training sessions of 30-min duration. Initially, each response on either lever was reinforced. After responding occurred reliably on both the left and right levers, discrimination training began. One lever was designated the muscimol lever and the other the saline lever. On days when muscimol (1.0 mg/kg) was administered intraparationally (IP), only responding on the muscimol lever was reinforced. On days when saline (1.0 ml/kg IP) was administered, only responding on the saline lever was reinforced. The response requirement for reinforcement was increased gradually and independently for each lever to a fixed-ratio 32 (FR 32). Incorrect lever presses reset the ratio value on the correct lever. Rats received muscimol or saline injections according to a double-alternation schedule (i.e., saline, saline, muscimol, muscimol, saline, etc.). Following weighing and injections, rats were returned to their home cages and 15 min later placed in operant chambers just before the start of the session.

Acquisition Testing

Successful acquisition of the muscimol-saline discrimination was determined using 2-min test probes conducted after reliable responding under the FR 32 schedule of food reinforcement was established on both levers. During the 2-min test probes, responding on either the muscimol or saline lever was reinforced. Following these 2-min probes, responding on only the correct lever was reinforced for the remaining 28 min of the session. Test probes were conducted every fourth session provided that the following criteria were met in the three previous training sessions: completion of the first FR on the correct lever, greater than 90% correct lever-responding and response rates greater than one response per second. Test probes were conducted in an alternating sequence of saline, muscimol, saline, muscimol until subjects learned the discrimination. Successful performance during the probe was determined based on the same criteria just listed.

Substitution Testing

Test sessions were 30 min in duration and occurred on Tuesdays and Fridays if the animal had met the criteria listed above throughout the 30-min session on the preceding training day. During test sessions, responding on either lever was reinforced under the FR 32 schedule; responding on one lever reset the FR requirement on the opposite lever. Between test sessions (Monday, Wednesday, and Thursday) training was continued as above under the double alternation sequence.

The following drugs were evaluated in the order given: muscimol (0.03–3.0 mg/kg), THIP (1–10 mg/kg), diazepam (0.1–5.6 mg/kg), tiagabine (1.0–17.6 mg/kg), progabide (100–560 mg/kg), valproic acid (30–300 mg/kg), vigabatrin (100–

1000 mg/kg) and propofol (3–30 mg/kg). Muscimol and progabide were administered 15 min prior to the session. THIP, diazepam, tiagabine, and valproic acid were given 30 min, propofol 10 min, and vigabatrin 60 min prior to the session. All drug injections were given in a volume of 1 ml/kg. Not every subject was tested with every compound; however, each dose effect curve represents data for 5–12 subjects as described in the figure legends. Prior to and after the assessment of each dose–effect curve, control test sessions with muscimol (1.0 mg/kg) and saline were conducted.

Drugs

Muscimol hydrobromide and THIP hydrochloride (Research Biochemicals, Natick, MA) were dissolved in 0.9% NaCl. Diazepam (Elkin-Sinn, Cherry-Hill, NJ) was administered in 40% propylene-glycol, 10% ethanol, 5% sodium benzoate, and water vehicle with 1% benzyl alcohol and benzoic acid as preservatives. Tiagabine (Novo Nordisk A/S, Denmark) was dissolved in sterile water. Progabide (Synthelabo Reserche, Bagneux, France), valproic acid (sodium salt) (Sigma Chemical Co., St. Louis, MO), and vigabatrin (gamma-vinyl GABA) (Marion Merrell Dow, Cincinnati, OH) were suspended in 1% Tween 80 and dissolved in sterile water. Propofol (Research Biochemicals, Natick, MA) was dissolved in sterile water.

Analysis of Data

Acquisition and test data were treated separately. The total number of sessions required before successful completion of the four test probes was determined for each subject. For each 30-min test session, percentage of muscimol-lever responding and response rate were calculated. Group means (±SE) for the subjects were calculated for both the percentage of muscimol-lever responding and response rate. For test sessions where high drug doses suppressed a subject's response rate to less than 0.05 responses per second, the lever selection data were excluded from the group data, although response rate data for the session were analyzed. Furthermore, if responding failed to occur in a majority of the subjects tested, the lever selection data for that dose was not included in the graphical presentation. Whenever possible, ED_{50} values were calculated for those drugs that produced greater than 50% muscimol-lever responding. All ED_{50} (and 95% confidence limit) calculations were based on the linear portion of the mean dose–effect curve following a log10 transformation of dose and were performed using Statistical Analysis Systems Pharm/PCS version 4 (40). For the response rate data, drug effects were converted to percentage of control with the average of the response rate on the saline control test sessions conducted before and after each dose–response curve determination used as the control response rate.

RESULTS

Acquisition and Control Test Results

Acquisition of the muscimol/saline discrimination required an average of 117 training sessions (range 78–196). The stability of stimulus control by muscimol and saline injections during drug testing was examined via repeated control tests with 1 mg/kg muscimol and saline (Figs. 1–7, upper panels). Saline control tests always resulted in averages of less than 10% muscimol-lever responding and muscimol control tests nearly always resulted in greater than 90% muscimol-lever responding. Rates of responding on control test sessions throughout the study were similar for muscimol and saline test days, averaging 1.9 and 2.1 responses per second for muscimol and saline, respectively (Figs. 1–7, lower panels).

Other doses of muscimol produced a dose-dependent increase in muscimol lever responding (Fig 1). Regression analysis of the muscimol dose–effect curve yielded an ED_{50} value of 0.47 mg/kg (95% CL = 0.36–0.61). Muscimol also produced dose-dependent decreases in rates of responding up to 3 mg/kg, which reduced rates to 30% of control values (Fig. 1, lower panel). THIP also produced dose-dependent increases in muscimol-lever responding with full substitution at 7.5 and 10 mg/kg and an ED_{50} value of 3.6 mg/kg (95% C.L. 2.3–5.6) (Fig. 1, upper panel). Additionally, dose-related decreases in rates of responding were observed with THIP (Fig. 1, lower panel). For THIP, doses that produced maximal levels of muscimol-lever responding also resulted in response rate decreases.

Valproic acid completely failed to substitute for muscimol except at one dose (170 mg/kg), which produced an average of 53% muscimol-lever responding (Fig. 2). This dose fully substituted in three subjects, produced intermediate levels of responding in two rats, saline-lever responding in three subjects, and completely suppressed responding in the remaining ani-

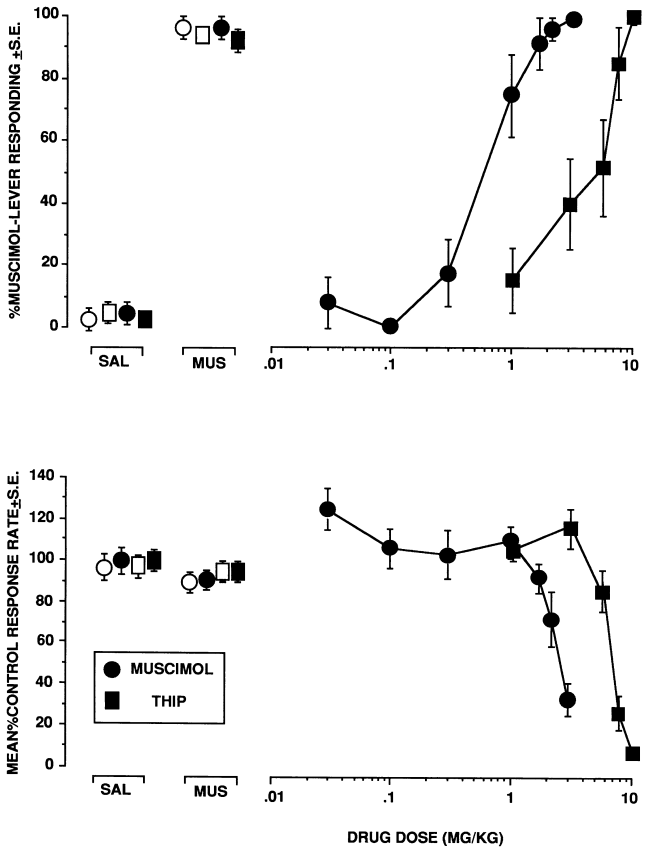

FIG. 1. Dose–effect curves for muscimol (●) (n = 12) and THIP (■) (n = 10) in muscimol-trained rats. The points above SAL and MUS represent control tests with saline and muscimol prior to (open symbols) and after (closed symbols) testing doses of muscimol (0.03–3.0 mg/kg) and THIP (1–10.0 mg/kg). Percentage of muscimol lever responding is shown in the upper panel; rates of responding are shown in the lower panel.

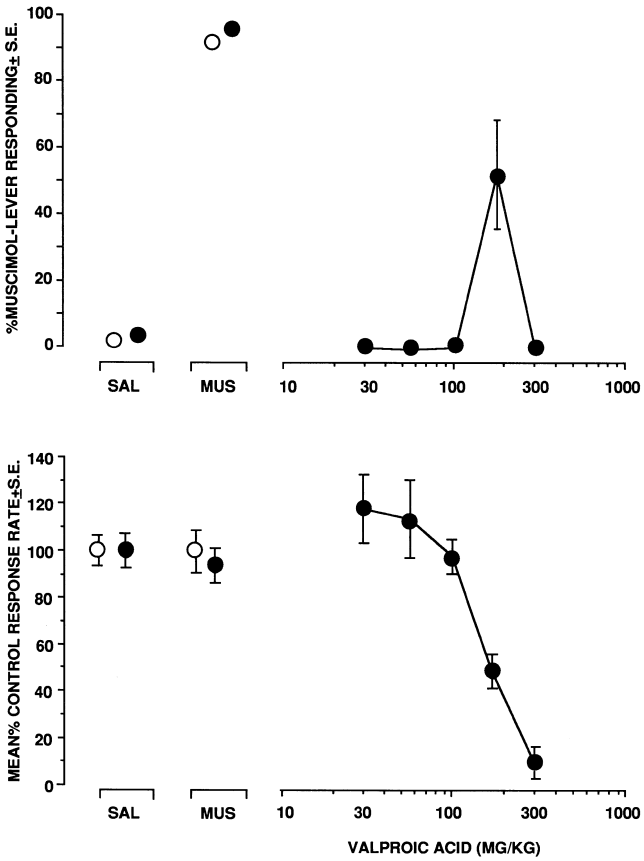

FIG. 2. Dose–effect curves for valproic acid in muscimol-trained rats (n = 9). The points above SAL and MUS represent control tests with saline and muscimol prior to (open symbols) and after (closed symbols) testing doses of valproic acid (30–300 mg/kg). Percentage of muscimol responding is shown in the upper panel; rates of responding are shown in the lower panel.

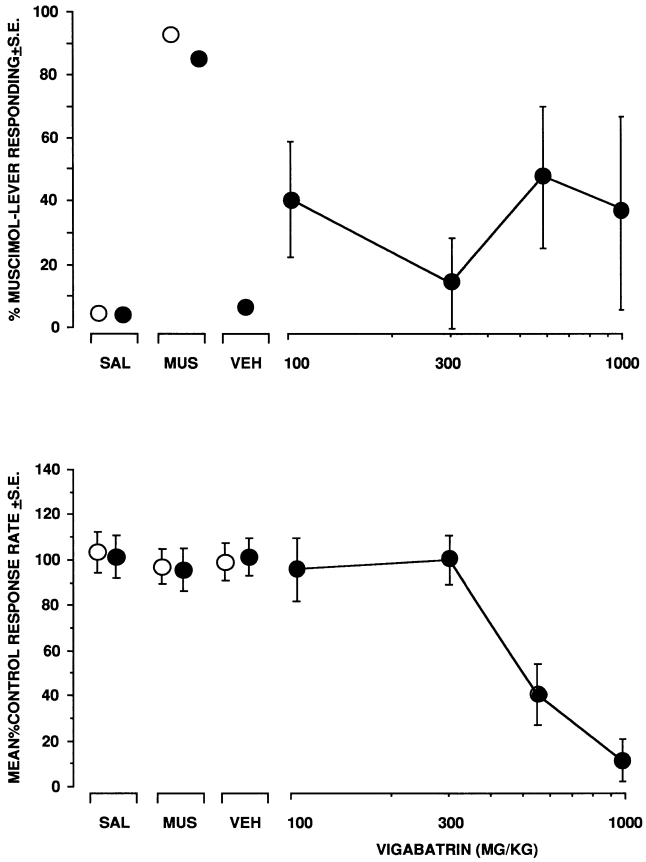

FIG. 3. Dose–effect curves for vigabatrin in muscimol-trained rats (n = 8). The points above SAL, MUS, and VEH represent control tests with saline, muscimol, and vehicle prior to (open symbols) and after (closed symbols) testing doses of vigabatrin (100–1000 mg/kg). Percentage of muscimol responding is shown in the upper panel; rates of responding are shown in the lower panel.

mal. Although the 170 mg/kg dose resulted in partial substitution, response rate was decreased to 46% of baseline.

Vigabatrin yielded a maximal 46% muscimol-lever responding at 560 mg/kg (Fig. 3, upper panel); however, all doses tested resulted in some muscimol-lever responding in a few subjects. For example, the 560 mg/kg dose fully substituted in two rats, produced intermediate muscimol-lever responding in one rat, occasioned only saline-lever responding in three subjects, and in the remaining two subjects response rates were completely suppressed. Although 560 mg/kg yielded the greatest level of substitution, it was associated with a decrease in response rates to 39% of saline control values. Vigabatrin testing produced dose-dependent decreases in response rate (Fig. 3, lower panel).

Of the drugs tested other than muscimol and THIP, progabide occasioned the highest level of muscimol-lever responding, with 71% muscimol-lever responding at 300 mg/kg (Fig. 4, upper panel). At this dose, five subjects responded solely on the drug lever and the remaining two rats responded on the saline lever. No response rate decreasing effects were observed at this dose; however, a large decrease was obtained at 560 mg/kg (Fig. 4, lower panel).

Tiagabine produced no greater than 48% muscimol-lever responding at the 17.6 mg/kg dose (Fig. 5, upper panel) . This resulted from three subjects responding on the muscimol lever, four animals responding almost exclusively on the saline lever, and the remaining three rats showing complete response rate suppression. Only minimal dose-related decreases in mean rates of responding were produced (Fig. 5, lower panel). However, in a subset of subjects dose-dependent decreases in responding were observed. Of the five rats in which decreases in response rates occurred, only two responded primarily on the muscimol-associated lever at any dose.

Figure 6 shows the dose–response curve for propofol. Lower doses of propofol (3–10 mg/kg) produced primarily saline-lever responding with mean values of less than 25% muscimol-lever responding. Maximal levels of approximately 53% muscimol-lever responding occurred at the 30 mg/kg dose, representative of two rats responding on the drug-associated lever, two subjects responding on the saline lever and the remaining subject responding on both. Although the greatest level of muscimol-lever responding occurred at the highest dose tested, this suppressed rates of responding to 38% of saline control values.

Diazepam yielded a maximum mean of 50% muscimol-lever responding at the 3 mg/kg dose, which also decreased rates of responding (Fig. 7) to 41% of baseline control values. Of the 11 subjects tested, 5 responded solely on the muscimol

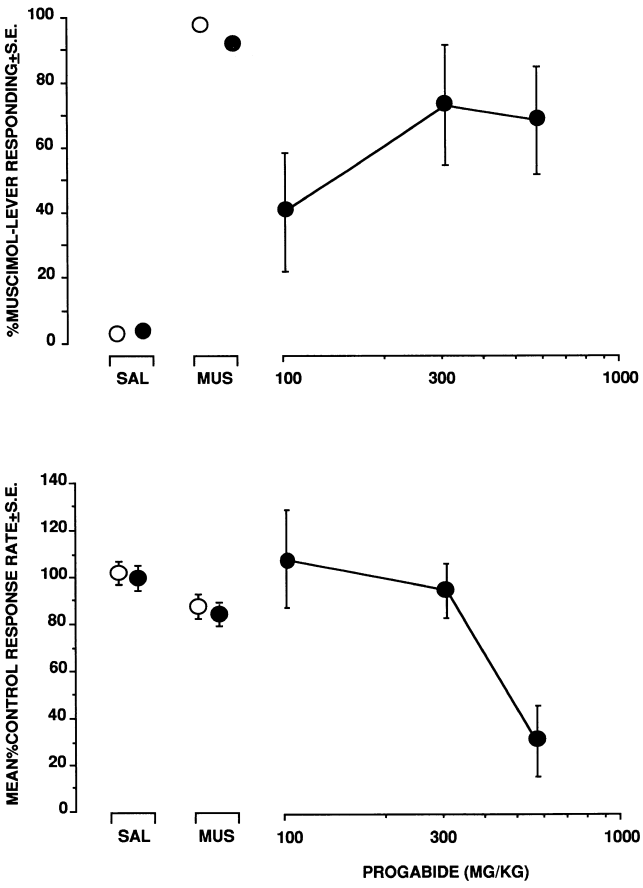

FIG. 4. Dose–effect curves for progabide in muscimol-trained rats (n = 7). The points above SAL and MUS represent control tests with saline and muscimol prior to (open symbols) and after (closed symbols) testing doses of progabide (100–560 mg/kg). Percentage of muscimol responding is shown in the upper panel; rates of responding are shown in the lower panel.

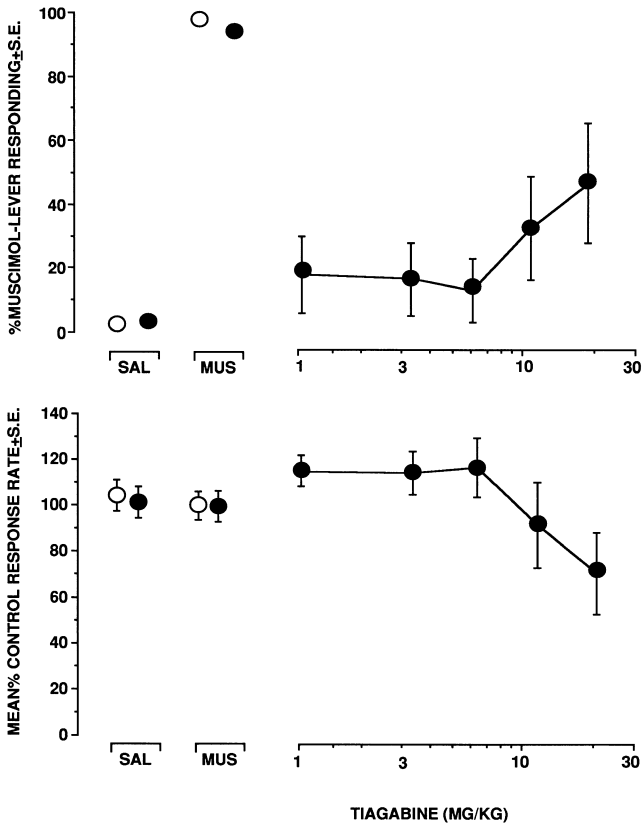

FIG. 5. Dose–effect curves for tiagabine in muscimol-trained rats (n = 10). The points above SAL and MUS represent control tests with saline and muscimol prior to (open symbols) and after (closed symbols) testing doses of tiagabine (1.0–17.6 mg/kg). Percentage of muscimol responding is shown in the lower panel; rates of responding are shown in the lower panel.

lever, 5 responded almost entirely on the saline lever, and 1 rat failed to respond at all.

DISCUSSION

The principal finding of this study is that complete substitution for muscimol in a drug discrimination study was produced only by THIP, another direct $GABA_A$ agonist. Other types of GABA agonists that were studied produced, at best, partial substitution, and their profile of discriminative stimulus and response rate effects could be easily distinguished from those of muscimol and THIP. Drugs that failed to produce full substitution for muscimol in this study were valproic acid, vigabatrin, progabide, tiagabine, propofol, and diazepam, representing a diverse array of site-selective GABA agonists. In a previous study of muscimol discrimination (16), similar results were obtained with midazolam, pentobarbital, baclofen, and phencyclidine, providing even further evidence of the pharmacological selectivity of the muscimol stimulus. These results do not appear to be due to an action of muscimol other than on $GABA_A$ receptors, because, in addition to the full substitution obtained with THIP, bicuculline was found to antagonize muscimol's discriminative stimulus effects (16). Therefore, it is becoming clear that muscimol produces a distinct discriminative stimulus, not shared by GABA agonists that do not interact directly and selectively with the $GABA_A$ site on the GABA receptor complex.

In general, full substitution occurs with benzodiazepines in barbiturate-trained animals, but not with barbiturates in benzodiazepine-trained animals (2–5,9,21,39). Commonalties have also been reported in the discriminative stimulus effects of anesthetic steroids, benzodiazepines and barbiturates (9,21) and among all these drugs and ethanol (11,24,38). This conclusion is consistent with the quite different overall profile of pharmacological effects seen between direct GABA agonists and barbiturates and benzodiazepines (25). Perhaps this should not be surprising, considering that the mushroom containing muscimol is considered psychotomimetic and muscimol produces a profile of acute intoxication and cognitive impairment that differs substantially from that of typical CNS depressant drugs (41).

Acquisition of the muscimol-saline discrimination was relatively slow. An average of 117 training sessions was needed before subjects reached criterion. There was also a consider-

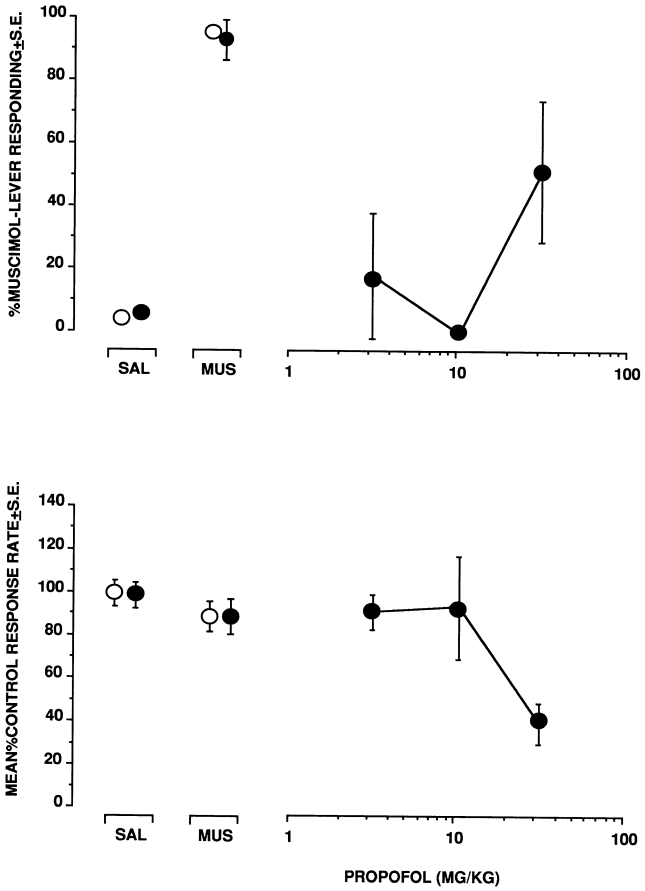

FIG. 6. Dose–effect curve for propofol in muscimol-trained rats (n = 5). The points above SAL and MUS represent control tests with saline and muscimol prior to (open symbols) and after (closed symbols) testing doses of propofol (3.0–30.0 mg/kg). Percentage of muscimol responding is shown in the upper panel; rates of responding are shown in the lower panel.

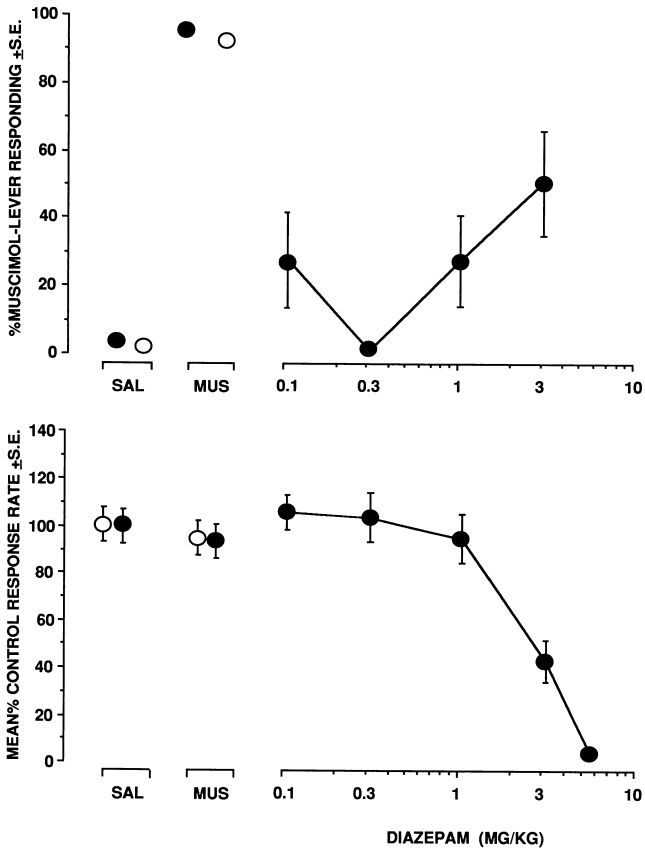

FIG. 7. Dose–effect curve for diazepam in muscimol-trained rats (n = 11). The points above SAL and MUS represent control tests with saline and muscimol prior to (open symbols) and after (closed symbols) testing doses of diazepam (0.1–5.6 mg/kg). Percentage of muscimol responding is shown in the upper panel; rates of responding are shown in the lower panel.

able range of training session needed, from 58 to 196. These acquisition data are very comparable to what was reported earlier (16), where an average of 91 sessions were needed, and three subjects never acquired the discrimination. Under similar training conditions, ethanol, barbiturate, and benzodiazepine discriminations are usually acquired much more rapidly [e.g. (4,14,15)]. In our previous study (16), it was also difficult to maintain the muscimol discrimination once it was acquired, but that was not the case in this group of rats. Control test sessions with saline and the training dose of muscimol (1 mg/kg) interspersed throughout the period of substitution testing provided evidence for good stimulus control. Only occasionally did an animal from the group respond predominately on the incorrect lever.

The results with THIP are a direct replication of our previous study in a different group of subjects. We have reported that THIP produced muscimol-lever selection in five of six rats tested (16). Similar to substitution tests with muscimol, full muscimol-appropriate responding occurred only at THIP doses that also produced severe rate decreasing effects. Thus, the ability of THIP and muscimol to produce muscimol-like discriminative stimulus and rate reducing effects appear very similar.

It might have been expected that GABA agonists that act to increase synaptic levels of GABA would have produced muscimol-like discriminative stimulus effects because all of these drugs would result in direct activation of the GABA site on the GABA–receptor complex, but such was not the case. Neither the GABA uptake inhibitor tiagabine nor vigabatrin or valproic acid, which inhibit GABA metabolism, produced full substitution for muscimol. Of course, these drugs would not be selective for activation of $GABA_A$ receptors, and it has been shown that the $GABA_B$ agonist baclofen fails to fully substitute for muscimol (16). Previous studies have also found differences in the discriminative stimulus effects of presynaptic and postsynaptic GABA agonists. As in muscimol-trained rats, tiagabine produces only partial substitution in rats trained with pentobarbital (15). Although little is known about the discriminative stimulus effects of vigabatrin, it has been shown not to substitute for pentobarbital in rats (15). Valproic acid has produced somewhat mixed results when compared to barbiturates and benzodiazepines in drug discrimination studies. Grech and Balster (15) reported full substitution for pentobarbital in rats, but only at a dose that produced greater than 50% decreases in rates of responding. In pigeons, valproic acid fails to substitute for pentobarbital (21).

Valproic acid has been found to produce partial substitution for midazolam (34), and like barbiturates and benzodiazepines, to antagonize the stimulus effects of pentylenetetrazol (26). Taken together, drug discrimination studies with presynaptic GABA agonists show that they can usually be distinguished from all the kinds of postsynaptic $GABA_A$ agonists that have so far been studied.

Progabide produced the greatest level of muscimol-lever responding of any of the drugs tested with the exception of THIP. Full generalization from muscimol to progabide occurred in five of the seven subjects tested at doses that did not alter the rate of fixed-ratio responding. The remaining two rats responded primarily on the saline lever at all doses. In pentobarbital-trained rats dose-dependent partial substitution was observed; however, doses that occasioned maximal pentobarbital-lever selection also produced substantial response rate reduction.

Because progabide is a prodrug for a compound that is reported to interact directly with the GABA site on the $GABA_A$ receptor (27,42), it may not be surprising that it has some muscimol-like discriminative stimulus effects. The fact that progabide produces a pattern of results that could be distinguished from that of muscimol, supports the idea that it may have GABA agonist effects via other actions as well as direct receptor activation.

Another postsynaptic $GABA_A$ agonist propofol, resulted in partial substitution for muscimol, but only at the highest dose, which was also associated with response rate suppression. In vitro evidence suggests that propofol has a site of action distinct from that of benzodiazepines (19) and that may involve interactions with the $GABA_A$ receptor at a distinct recognition site (10,33). Therefore, the partial substitution of propofol in muscimol-trained rats combined with the in vitro data further support the notion that the cellular site at which drugs act to enhance GABAergic function can produce differences in their discriminative stimulus effects.

In conclusion, it is reported in now a second study that rats can be trained to discriminate muscimol from saline. Although the discrimination is difficult to acquire, it can be quite stable over an extended period of testing. It appears that the discriminative stimulus properties of muscimol are quite specific for direct acting $GABA_A$ agonists acting at the GABA site, because in this study and a previous one (16), drugs representing other classes of GABA agonists did not produce full substitution. The most interesting of the new drugs tested were the GABA uptake inhibitor tiagabine, and the inhibitors of GABA metabolism, vigabatrin and valproic acid. They too are likely to possess unique discriminative stimulus effects because they do not cross generalize with barbiturates or muscimol. Drug discrimination procedures are useful to distinguishing among the behavioral effect of classes of GABA agonists.

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