Imidazenil prevention of alprazolam-induced acquisition deficit in patas monkeys is devoid of tolerance

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The partial allosteric modulators (PAMs) of γ-aminobutyric acid-gated Cl^- current intensities at γ-aminobutyric acid type A receptors have high affinity but low intrinsic efficacy on benzodiazepine recognition sites. Unlike the full allosteric modulators (FAM), like alprazolam, triazolam, and diazepam, PAMs are virtually devoid of unwanted side effects, including tolerance. Imidazenil (IMD) is a PAM that elicits potent anxiolytic and anticonvulsant actions in rodents and nonhuman primates and retains its anticonvulsant and anxiolytic effects, even in rodents that are tolerant to FAMs. IMD antagonizes the side effects of FAMs in rodents and nonhuman primates. Using patas monkeys and a multiple schedule with repeated acquisition and performance of chain responses, we report that IMD administration for 17 days antagonized without showing tolerance ALP-induced disruption of acquisition.

Currently prescribed anxiolytic drugs that bind to the benzodiazepine recognition sites (BZ-Rs) expressed by γ-aminobutyric acid type A ($GABA_A$) receptors act as full allosteric modulators (FAMs) of GABA-gated Cl^- current intensities (1, 2). Frequently, doses of FAMs in the range of those prescribed to relieve anxiety induce a number of unwanted side effects, including cognitive deficit, sedation, and ethanol or barbiturate potentiation; in addition, tolerance and dependence liability may occur within a few weeks of continued therapy (for a review, see ref. 3). Very likely, the cause for the high incidence of unwanted effects, including tolerance, does not reside in the indiscriminate modulation of several $GABA_A$ receptor subtypes elicited by FAMs but in their tendency to maximize the amplification of GABA-gated Cl^- current intensities, even when given in the range of clinically recommended doses (1–4).

The reports that postmortem brains of schizophrenia and bipolar disorder patients with psychosis exhibit a marked down-regulation of glutamic acid decarboxylase 67 (GAD_{67}) (5, 6) has prompted several investigators to test the action of FAM benzodiazepines (BZDs) (the only anxiolytic BZDs currently approved for clinical use) in the treatment of these psychosis (for a review, see ref. 7). These BZDs cause a remission of the negative symptoms associated with psychosis, but their beneficial effects last for only a few weeks, very likely because they are interrupted by the onset of tolerance (7).

The discovery by Haefely *et al.* (8) of a BZ-Rs ligand (termed bretazenil) with high affinity but low intrinsic efficacy, acting as partial positive allosteric modulator (PAM) of GABA-gated Cl^- current intensities at a great variety of recombinant $GABA_A$ receptor subtypes (9–11), has provided new insights into the development of a new generation of BZ-Rs ligands with therapeutic potential in the treatment of psychosis (8). So far, PAMs, including bretazenil and the successively synthesized imidazenil (IMD), have been tested in rodents, dogs, and nonhuman primates, and bretazenil also was tested in humans affected by schizophrenia and shown to be virtually devoid of unwanted side effects, such as amnesia, sedation or tolerance liability, even at doses 2–3 times greater than those eliciting potent anxiolytic, antipanic, and anticonvulsant actions (for a review, see refs. 1 and 12). Probably PAMs pharmacological profile is not determined by the selectivity for a specific $GABA_A$ receptor subtype but rather by their modest amplification of GABA-gated Cl^- current intensities at every $GABA_A$ receptor subtype (1, 11).

A plausible explanation for why PAMs may produce anxiolytic or anticonvulsant action without producing unwanted side effects and signs of tolerance, even after protracted administration of high doses, may relate to their ability to maintain a sufficient $GABA_A$ receptor responsiveness to changes in rates of quantal release of GABA from presynaptic terminals of inhibitory synapses, modulating neuronal circuits regulating vigilance, cognition, and other cortical functions. Hence, PAMs, while positively modulating $GABA_A$ receptor, preserve a buffering flexibility that maintains the $GABA_A$ receptor's ability to amplify GABA-gated Cl^- current intensities in a manner related to the changing amounts of GABA released from nerve terminals. It is important to keep in mind that, among various types of cortical neurons, the GABAergic neurons exhibit the highest firing rates that are precisely modulated to change the characteristic patterns of synchronous firing of pyramidal neurons during the columnary activity that codifies cortical functional output (1, 13). In contrast, the amplification of GABA-gated Cl^- current intensities elicited by FAMs or selective allosteric modulators (SAMs) is maximized by the therapeutic doses of these two classes of BZ-R agonists. This maximal amplification of GABAergic tone in the neuronal circuits targeted by FAMs or SAMs creates a hindrance to respond to graded quantal release of GABA regulated by nerve impulses, thus resulting in alteration of columnary activity of pyramidal neurons that presumably results in cortical dysfunction.

The present report shows that, in monkeys working on a complex behavioral task of repeated acquisition and performance of response chain, alprazolam (ALP) (a FAM of the BZ-Rs) given in doses similar to triazolam doses that elicit anxiolytic responses in monkeys (*Saimiri Sciureus*) (14, 15) produced dose-related disruption of acquisition with little or no effect on performance. In contrast, IMD given in doses multiple of those that elicit anxiolytic-like action (16, 17) not only failed to disrupt acquisition or performance but also attenuated the disruptive effects of ALP on acquisition (14). In the present study, we investigated whether, in monkeys working on a complex behavioral paradigm of repeated acquisition and performance of response chain, protracted administration of IMD is associated with tolerance to its inhibitory action against ALP-induced acquisition impairment.

Abbreviations: PAM, partial allosteric modulator; FAM, full allosteric modulator; SAM, selective allosteric modulator; ALP, alprazolam; IMD, imidazenil; BZD, benzodiazepine, BZ-Rs, benzodiazepine recognition site; GABA, γ-aminobutyric acid; GAD_{67}, glutamic acid decarboxylase 67; DZ, diazepam.

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Materials and Methods

Subjects. Two female and one male patas monkeys (*Erythrocebus patas*) housed individually with free access to water served as subjects. They were maintained at about 90% of their free-feeding weights on a diet consisting of Purina Monkey Chow, fresh fruits, vitamins fed in their home cages, and Noyes banana-flavored pellets received during experimental sessions. All three subjects had extensive experience with the behavioral procedure used and had been exposed to a variety of drugs in the past but were drug-free for at least 8 mo before beginning the present study.

Apparatus and Behavioral Procedure. The apparatus and the behavioral baseline used have been described (18, 19). To characterize drug effects, a multiple schedule with acquisition and performance components served as the behavioral baseline. During the acquisition component, the subject task was to learn a different four-response chain during each daily session by pressing the correct key in the presence of different combinations of colors and geometric forms. The four-response chain was maintained by food presentation under a fixed-ratio (FR5) schedule; i.e., every fifth completion of the chain produced a food pellet (500 mg) when the pilot lamp was pressed. In the performance component, the four geometric forms were projected on a green background and the four-response chain remained the same (left-center-left-right) from session to session. In all other aspects (e.g., FR5 schedule), the performance and acquisition components were identical.

Each daily session began with the acquisition component and alternated with the performance component after 10 reinforcements (food pellets) or 15 min, whichever occurred first. A 5-s timeout, during which all stimuli were off and responses had no programmed consequences, separated consecutive components. Each daily session was terminated after a fixed number of pellet deliveries or reinforcements (60 for females and 100 for the males) or 2 hr, whichever occurred first.

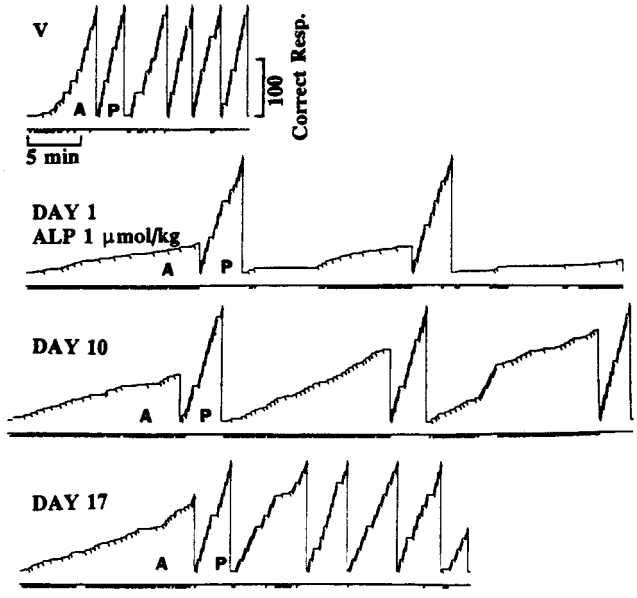

Fig. 1. Cumulative records showing the within-session pattern of responding for monkey A during representative vehicle (V) session and sessions preceded by administration of 1.8 μmol/kg of ALP on days 1, 10, and 17 of a 17-day ALP daily treatment. Each record represents a complete session (reinforcements), except on days 1 and 10 of ALP treatment, which show the first 60 min of the respective treatments during the 2-hr sessions. The response pen stepped with each correct response and was deflected downward each time food was presented. Errors are indicated by the event pen (below each record), which was held down during each timeout. The event pen was deflected and the response pen reset each component of the multiple schedule changed. Each daily session began with a different four-response chain in the acquisition component (A) and then alternated with a performance component (P) after 10 reinforcements or 15 min, whichever occurred first.

Drug Testing. *Single-dose testing.* Either 0.56 or 1 μmol/kg of ALP was administered orally 30 min presession one or two times a week. ALP was suspended in a few drops of Tween 80, and this mixture was diluted (0.5 ml/kg) with 15 ml of Hawaiian fruit punch. The effects of ALP were tested on Tuesdays and Fridays, and vehicle sessions (control) were recorded on Thursdays. We selected these two doses because in an earlier work they were shown to selectively disrupt acquisition (14, 20).

Repeated-dose testing. When IMD was administered for protracted time periods, 0.25, 0.5, and 1.25 μmol/kg of IMD,

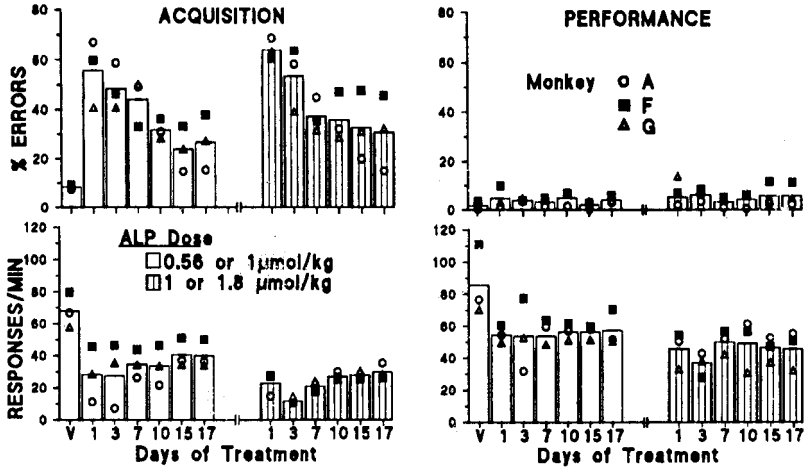

Fig. 2. Effects of repeated administration of 0.56 or 1 μmol/kg (open bars) and 1 or 1.8 μmol/kg (hatched bars) of ALP alone, on response rate and percent errors in each component of the multiple schedule for three subjects. The bars indicate the mean values for at least 10 vehicle sessions and single determinations during repeated daily administration of ALP. The symbols represent the values for the individual subjects after the treatment with the respective doses of ALP. Monkeys A and G received daily administration of 1 (open bars) and 1.8 (hatched bars) μmol/kg, whereas monkey F received 0.56 (open bars) and 1 (hatched bars) μmol/kg of ALP. Note that monkey F received lower doses of ALP because this subject was more sensitive to the effects of ALP.

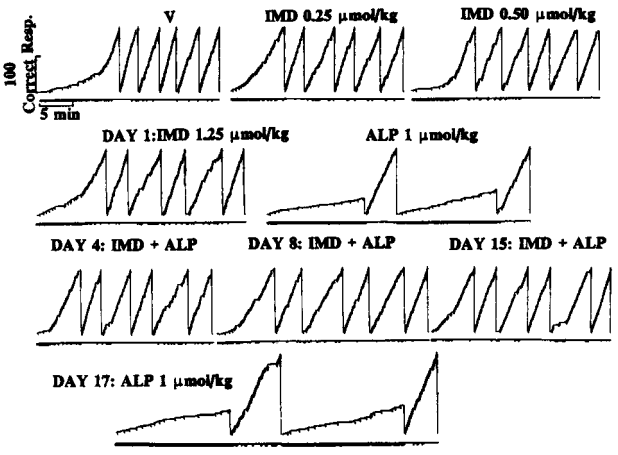

Fig. 3. Cumulative record showing within-session pattern of responding for monkey G during a representative vehicle (V) session and sessions preceded by the administration of 0.25, 0.5, and 1.25 μmol/kg of IMD or 1 μmol/kg ALP alone, and a combination of this dose of ALP with 1.25 μmol/kg IMD on days 4, 8, and 15 of repeated IMD (1.25 μmol/kg) treatment. The same single dose of ALP was administered alone on day 2 after discontinuation of a 17-day repeated IMD treatment. The response pen stepped with each correct response and was deflected downward each time food was presented. Errors are indicated by the event pen (below each record), which was held down during each timeout. The event pen was deflected and the response pen reset each time the component of the multiple schedule changed. Each session began with an acquisition component (A) and then alternated with a performance component (P) after 10 reinforcements or 15 min, whichever occurred first.

prepared as described for ALP, were given orally once daily 60 min presession. When IMD and ALP were given in combination on days 4, 8, and 15 of a once daily IMD treatment, they were administered 60 and 30 min presession, respectively.

During the administration of single daily ALP doses, monkeys G and A received 1 μmol/kg, whereas monkey F received 0.56 μmol/kg, of ALP once daily 30 min presession for 17 days. The monkeys were kept 8 wk drug-free before receiving a treatment with the higher doses of ALP. Monkeys G and A received 1.8 μmol/kg and monkey F received 1 μmol/kg of ALP for another 17 days. During the daily (Monday through Sunday) repeated administration (IMD or ALP) studies, sessions were recorded on Monday through Friday only. There was a 3-wk drug-free interval between IMD and ALP protracted daily treatment.

Data Analysis. The effects of drugs on acquisition and performance in the multiple schedule were analyzed in terms of the overall response rate (responses per min) and the overall accuracy determined by the percentage errors [(incorrect/correct + incorrect responses) × 100] in each component. The changes induced by drug treatment were analyzed by comparing drug and vehicle sessions in the same monkey with the control sessions (vehicle sessions) range of variability. Each subject served as its own control, and ranges of response variability were established during vehicle sessions. Drug effects were considered significant to the extent that the data for a given dosage or drug fell outside of the ranges of variability established during vehicle sessions.

Results

Effects of Repeated Daily Doses of ALP on Acquisition. Fig. 1 illustrates a representative within-session pattern of responding for monkey A after vehicle and a 17-day treatment schedule with ALP (1 μmol/kg). During vehicle session (Fig. 1 *Left*), the subject acquired the new four-response chain in the first acquisition component, as shown by a decrease in the number of errors and an increase in errorless completions of the four-response chain. When 1 μmol/kg of ALP was administered, it decreased response rate in both acquisition and performance and selectively produced a large increase in percent errors in acquisition, whereas the accuracy of responding in the performance component was relatively unaffected. As shown in Fig. 1, on the first day of treatment, ALP almost eliminated responding in the second and third acquisition components and produced a large error-increasing effect in the first acquisition component. The error-increasing effects of ALP decreased after subsequent daily treatments with this ALP dose. Note also that the number of correct responses in acquisition (increased with subsequent repeated daily administration of ALP) becomes evident after 9 days of ALP administration. After 17 days of ALP treatment, the four-response chain was acquired toward the end of the first acquisition component of this session; in fact, the subsequent acquisition components were almost errorless. Overall, after 16 days of repeated treatment with ALP, the within-session pattern of responding was identical to the control conditions, indicating the development of tolerance to the error-increasing effects of ALP during acquisition.

Fig. 2 shows the effects of repeated administration of 0.56 or 1 μmol/kg (open bars) and 1 or 1.8 μmol/kg (hatched bars) of ALP on response rate and percent errors in three monkeys. The bars represent the average response rate and percent errors, whereas the points represent the individual subject variable distributions. All three monkeys showed a similar control percent errors in both acquisition and performance, whereas there were wide individual differences in response rates for the three monkeys in both acquisition and performance. The rates of responding were generally higher during the performance component. ALP produced comparable dose-dependent decreases in overall response rate in both components of the multiple schedule in all three subjects. The onset of tolerance to the rate-decreasing effects of ALP was slow in both components in all three monkeys. In contrast to its effects on response rate, ALP had a selective effect on percent errors. That is, ALP elicited a dose-dependent increase in percent errors in the acquisition component in all three subjects, whereas the performance component was virtually unchanged by ALP. Note also that there was differential sensitivity to the error-increasing effects of ALP among the subjects. Monkey A was more sensitive to the error-increasing effects than monkeys F and G, and monkey F was more sensitive than monkey G. In contrast to the rate-decreasing effects, the error-increasing effects of ALP showed rapid tolerance. Overall, tolerance to the error-increasing effects of ALP appeared on day 3 and reached a ceiling on day 15 of the 17 days of repeated ALP treatment.

IMD Prevention of ALP-Induced Acquisition Disruption. Fig. 3 depicts a representative within-session pattern of responding in monkey G after vehicle, 1 μmol/kg of ALP, 0.25, 0.50, and 1.25 μmol/kg of IMD alone, and a combination of 1 μmol/kg of ALP and 1.25 μmol/kg IMD after 4, 8, and 15 days of repeated IMD treatment. In the vehicle session, the errors were much more frequent in the first than in the subsequent acquisition components, during which acquisition of the new four-response chain was evidenced by a virtual errorless pattern of responding. Except when 1.25 μmol/kg of IMD was first administered on day 1, the within-session effects of IMD alone did not differ from that of vehicle session. The first dose of IMD caused a modest increase in percent errors and a slight decrease in the rate of responding in acquisition that disappeared after the second administration of this daily dose of IMD. In contrast to IMD, 1 μmol/kg of ALP produced a large error-increasing effect in the acquisition component throughout the session, without affecting the accuracy of responding in the performance. The record for the within-session com-

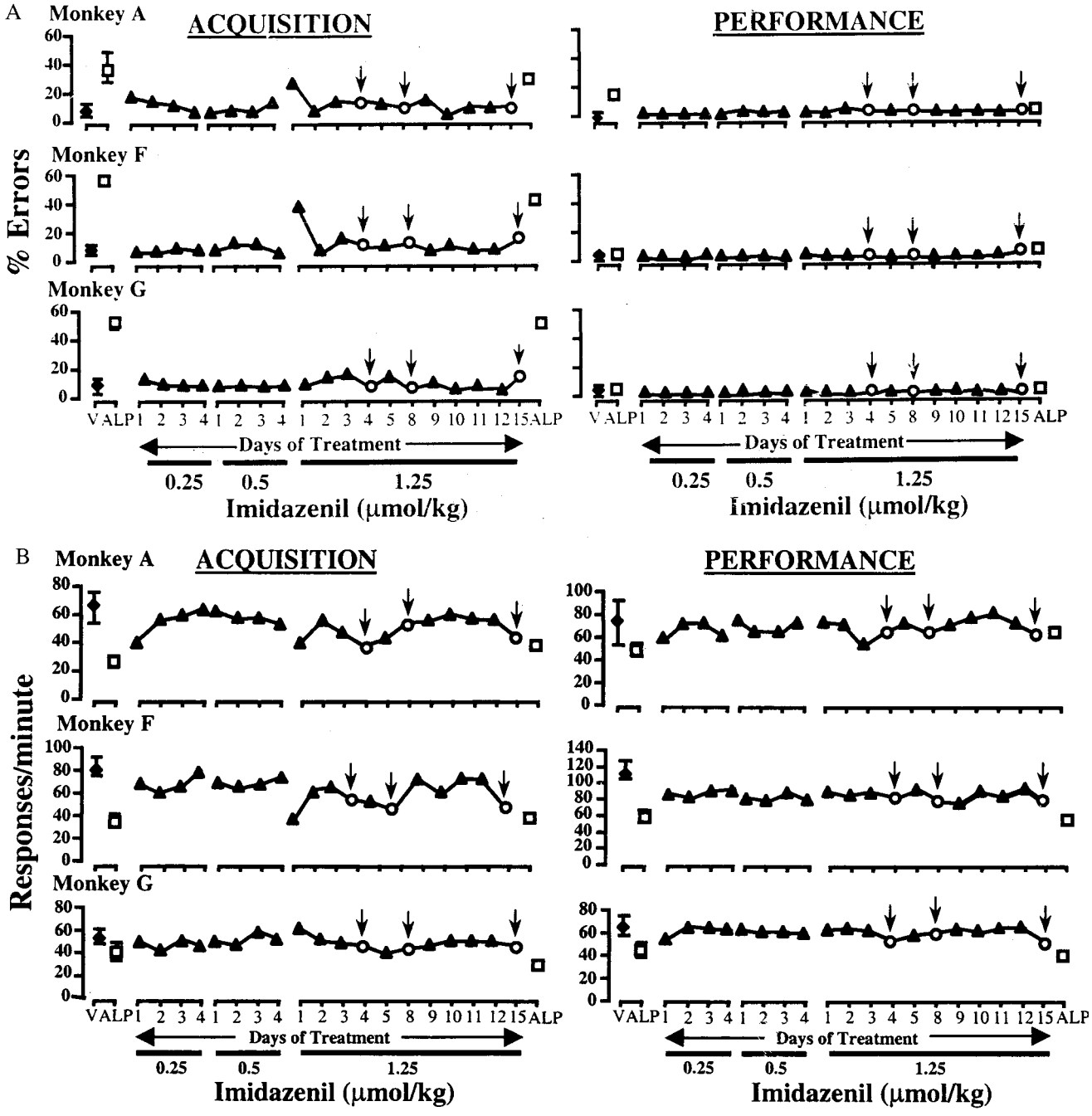

Fig. 4. (*A*) Effects of repeated oral administration of 0.25, 0.5, or 1.25 μmol/kg of IMD or 0.56 and 1 μmol/kg of ALP alone, and a combination of ALP with IMD during repeated administration of 1.25 μmol/kg of IMD on percent errors in each component of the multiple schedule for three subjects. Points and vertical lines at V and ALP indicate the mean and range for at least 10 vehicle (◆) and two ALP (□) sessions, respectively. The points in the time effect curves without vertical lines indicate single determinations of the effects of IMD alone (▲) or in combination (○, arrows) with a single dose of ALP. Monkeys A and G received 1 μmol/kg and Monkey F received 0.56 μmol/kg of ALP. All monkeys received repeated administration of increasing doses (0.25, 0.5, and 1.25 μmol/kg) of IMD for 4, 4, and 15 days, respectively. (*B*) Effects of repeated oral administration of 0.25, 0.5, and 1.25 μmol/kg of IMD and a single 0.56 or 1 μmol/kg of ALP alone, and a combination of ALP with IMD during repeated administration of IMD (1.25 μmol/kg) on response rates in each component of the multiple schedule for three subjects. All drug doses, symbols, keys, and schedule of treatments and subjects are the same as in *A*.

bination of IMD and ALP shows that the same dose of IMD that did not produce significant behavioral effects when administered alone, completely antagonized the increased percent errors of a single dose of ALP, even after 4, 8, and 15 days of repeated IMD (1.25 μmol/kg) administration. Moreover, ALP was still effective at disrupting acquisition 2 days after the discontinuation of 15 days of daily IMD treatment.

Fig. 4*A* shows the effects of repeated administration of 0.25, 0.50, and 1.25 μmol/kg of IMD and 0.56 or 1 μmol/kg of ALP alone, and a combination of 1.25 μmol/kg of ALP and 0.56 or 1 μmol/kg of ALP on days 4, 8, and 15 of repeated IMD administration on percent errors during acquisition and performance. In agreement with previous reports (14, 20), at the two doses tested, ALP produced a large increase in percent errors in

the acquisition component with little or no effect on percent errors in the performance component in all three subjects. In contrast to ALP, the lower doses (0.25 and 0.50 μmol/kg) of IMD tested had little or no effect on percent errors in either the learning or performance component. However, these doses of IMD antagonize the disruption of the acquisition component elicited by ALP (14, 15). In monkeys F and A, a small increase in percent errors in the acquisition component was elicited by the first oral dose of 1.25 μmol/kg of IMD, but this behavioral effect virtually disappeared when this dose was repeated the next day. Interestingly, repeated administration of this dose of IMD for 4, 8, and 15 days still antagonized the error-increasing effects of a single dose of ALP on the acquisition component in all three monkeys, suggesting a virtual lack of tolerance or accumulation of any active metabolite after repeated IMD administration. Moreover, ALP administered on day 2 after the abrupt discontinuation of a 15-day repeated IMD treatment was still effective at eliciting a large increase in percent errors in the acquisition component. Although not scored, no overt signs of withdrawal were observed after abrupt discontinuation of IMD-repeated administration.

Fig. 4*B* shows the effects of the treatment schedule in Fig. 4*A* on rate of responding in both the acquisition and performance components. In all three subjects, when ALP was administered alone, the overall response rates in both components decreased. In contrast, IMD either had no effect on rate of responding (monkey A) or it slightly decreased the response rate in acquisition (monkey F and G) on the first day of treatment with 1.25 μmol/kg. However, even these slight rate-decreasing effects were no longer evident during the second or third administration of the same dose. Again, similar to the accuracy of responding, repeated treatment with IMD for 4, 8, and 15 days attenuated the rate-decreasing effects of ALP in acquisition.

Discussion

Recent studies in mice with point mutation at position 101 of the α1GABA$_A$ receptor subunit have suggested that the sedative, anticonvulsant, and amnesic, but not the anxiolytic, actions of FAMs may depend on their interaction with GABA$_A$ receptors that include at least one α1 subunit in their assembly (4). From these data, one can infer that the GABA$_A$ receptors that include the α1 subunits are operative in the regulation of vigilance, neuronal excitability, and cognition (4). In discussing this matter, Wisden and Stephens (21) concluded that it should be possible to design SAMs of BZ-Rs that possess anxiolytic, antiepileptic, and antipanic actions, but are devoid of sedative and/or amnesic effects, by synthesizing drugs that selectively bind to GABA$_A$ receptors devoid of α1 subunits. However, it cannot be predicted *a priori* that these drugs are devoid of tolerance and/or dependence liability when administered in pharmacologically active doses for months. One of the obvious difficulties in finding SAMs active as antiepileptic, anxiolytic, or antipanic drugs, but devoid of tolerance liability, is that the neuronal circuits that are targets for such therapeutic actions may express GABA$_A$ receptors that include in their pentameric structures other α subunits. These GABA$_A$ receptors also may be susceptible to a maximal amplification by therapeutic doses of the new class of SAMs envisioned by Wisden and Stephens (21). Based on the experiences accumulated over the years with zolpidem (which given in small doses acts as a SAM at GABA$_A$ receptors that include the α1 subunit), we know that the maximal amplification of several GABA$_A$ receptor subtypes can occur with doses of zolpidem that are not much greater than the therapeutic doses selective for α1 GABA$_A$ receptor subunits. Furthermore, the low-tolerance liability that has been ascribed to this drug is with reference to its low sedative doses (22). Hence, one should test whether the new class of SAMs proposed by Wisden and Stephens shows a dose-dependent liability to tolerance, dependence, and all the side effects that are associated with a maximal amplification of GABA-gated Cl^- current intensities. Because of the modest amplification of GABA-gated Cl^- current intensities elicited by IMD, we expect that other PAMs may be devoid of tolerance liability.

IMD's affinity for the BZ-Rs expressed by GABA$_A$ receptors is several-fold higher than that of the FAM, ALP, or diazepam (DZ). When IMD is administered to rats in a dose 1/10 that of DZ, it antagonizes the sedative actions of DZ, and the duration of its antagonism against bicuculline-induced seizures lasts longer than that of DZ, as expected from IMD slow rate of metabolism (11, 23). In rats that received a treatment schedule of 180 days with equipotent anticonvulsant doses of DZ (35.6 μmol/kg) and IMD (2.3 μmol/kg) (24), the onset of DZ anticonvulsant tolerance action and the concurrent down-regulation of α1 and γ2 GABA$_A$ receptor subunit expression occurred within a few days of treatment (23–27). In contrast, tolerance to the anticonvulsant action and down-regulation of selected GABA$_A$ receptor subunit expression never occurred with IMD (23–28), even for doses three times those equipotent to DZ doses inducing tolerance. However, the tolerance liability of IMD in nonhuman primates was never tested. This study is important because the patas monkey position in the evolutionary tree allows us to predict whether or not one should expect IMD tolerance liability in human.

A multiple schedule with repeated acquisition and performance of response chains in monkeys has provided a suitable and stable baseline to evaluate the effects of repeated FAMs or PAMs treatment on acquisition. We previously have shown that pretreatment with a single dose of IMD attenuated the disruptive effects of ALP or triazolam (two FAMs) on acquisition (14, 15). The IMD effective doses for this attenuation are well within the range of those that inhibit conflict and convulsions in rats or monkeys (1, 11, 14, 16, 17, 23).

The present study consistently shows the potent rate-decreasing and the error-increasing effect of doses of ALP that are anxiolytic and occupy approximately 50% of BZ-Rs in rats (11). Because ALP disruptive action was present on acquisition but not on performance, one can infer that this drug preferentially affects acquisition, and at these doses, fails to disrupt memories reinforced by practice (for a review, see ref. 18). This preferential effect on acquisition allows the comparison of FAMs and PAMs on learning. It is important to note that similar testing of FAMs actions on cognition have been reported in human (29). In contrast to ALP, daily administration of IMD doses (Fig. 3) that are 10-fold greater than those reported to occupy 100% of brain BZ-Rs in rats (11), and to elicit anxiolytic effects in rats and monkeys (11, 16, 17, 23), produced only a modest and short-lasting disruption of acquisition. Moreover, this effect failed to occur on the second day of treatment (see Fig. 4*A*).

While ALP and its FAM congeners produce clear signs of withdrawal after abrupt discontinuation of protracted treatment (3, 23), no overt signs of withdrawal were observed in rodents or monkeys after the abrupt discontinuation of a protracted IMD treatment or after withdrawal precipitated by flumazenil (a potent antagonist of BZD action) administration (23).

Repeated IMD administration for more than 6 mo in rodents does not produce tolerance (24), and unlike the FAMs, IMD given in high doses for 3 wk, neither changes GABA$_A$ receptor expression density nor subunit assembly (24–26). Most interestingly, the behavioral responses elicited by IMD administration in rats tolerant to FAMs or SAMs remain unchanged (23, 25, 26). This finding is consistent with the hypothesis that SAM and FAM, by eliciting a maximal amplification of GABA-gated Cl^- current intensities, trigger the decrease expression of α1 and γ2

subunits of the $GABA_A$ receptor, which, in turn, is associated with the onset of tolerance (26).

An important aspect of interest is whether IMD has a value in the treatment of psychosis symptoms that might be related to the down-regulation of GABAergic transmission. One possible rationale might be that when GABA turnover is reduced during the down-regulation of GAD_{67} occurring in psychosis, PAMs might normalize the action of a below-normal release of GABA from nerve terminals. Thus, it would be of interest to investigate whether IMD can alleviate psychosis symptoms.

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