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RAPID COMMUNICATION

Low-Dose Alprazolam Augments Motor Activity in Mice

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LOPEZ, F., L. G. MILLER, D. J. GREENBLATT, S. M. PAUL AND R. I. SHADER. *Low-dose alprazolam augments motor activity in mice.* PHARMACOL BIOCHEM BEHAV **30**(2) 511–513, 1988.—The triazolobenzodiazepine alprazolam appears to have a unique clinical spectrum, and recent studies indicate unusual binding properties at the benzodiazepine receptor when assessed in vivo at low doses (0.02–0.05 mg/kg). To assess the behavioral activity of alprazolam at low doses, we examined open-field activity after one hour in mice treated with alprazolam, triazolam, and clonazepam. Following triazolam and clonazepam administration, open-field activity decreased in a dose-dependent fashion. In contrast, low doses of alprazolam resulted in an increase in open-field activity, whereas higher doses decreased activity. For all three drugs, activity was linearly related to receptor binding. Pretreatment with a dose of the benzodiazepine antagonist Ro15-1788 sufficient to fully occupy receptors had no effect on open-field activity, but when administered concurrently with alprazolam (0.05 mg/kg) prevented the increase in activity seen with alprazolam alone. Increased open-field motor activity represents a behavioral correlate to the increases in receptor binding seen with low-doses of alprazolam. Changes in activity appear to be mediated at the benzodiazepine receptor, since an antagonist prevents increased activity. These data suggest that the unique clinical effects of alprazolam may be due in part to unusual interactions with the benzodiazepine receptor.

Alprazolam Triazolam Open-field Benzodiazepine

THE triazolobenzodiazepines, a relatively new class of benzodiazepines, have gained widespread clinical use [4]. Members of this class include alprazolam, which is used as an anxiolytic and antipanic agent, with possible additional antidepressant activity [2,4], and triazolam, which is primarily used as a hypnotic [12]. Like other benzodiazepines, these drugs appear to exert their effects by binding to the central benzodiazepine receptor, and in turn modulating GABA binding at the $GABA_A$ receptor complex [5]. However, several prior studies using in vitro binding techniques [1,3] and our recent studies using in vivo binding methods [7] suggest that these compounds have unusual binding properties at the benzodiazepine site. In particular, we found that alprazolam at low doses augments benzodiazepine receptor binding, but at higher doses occupies the receptor as is observed with other typical benzodiazepines. To determine whether these unusual binding properties have pharmacodynamic correlates, we examined open-field activity in mice after treatment with alprazolam and triazolam.

METHOD

Male CD-1 mice, 6 to 8 weeks of age, were obtained from Charles River Laboratories (Wilmington, MA), maintained under a 12-hour light-dark cycle, and given laboratory chow and water ad lib. Alprazolam and triazolam were generous gifts from Upjohn, Inc. (Kalamazoo, MI), and Ro15-1788 and clonazepam were gifts from Hoffmann-LaRoche (Nutley, NJ).

Alprazolam, triazolam, Ro15-1788 and clonazepam were dissolved in propylene glycol and diluted with saline. Vehicle-treated mice received the highest concentration of propylene glycol used. All drugs were injected IP and behavioral testing was performed at one hour after injection.

Open-field activity was assessed during the morning hours (10–12 a.m.) using an Opto-Varimex (Columbus Instruments, Columbus, OH). This apparatus consists of two horizontal arrays of 15 infrared beams placed perpendicular to each other, and a vertical array of an additional 15 infrared beams set at 7.5 cm from the floor. The 3 mm diameter

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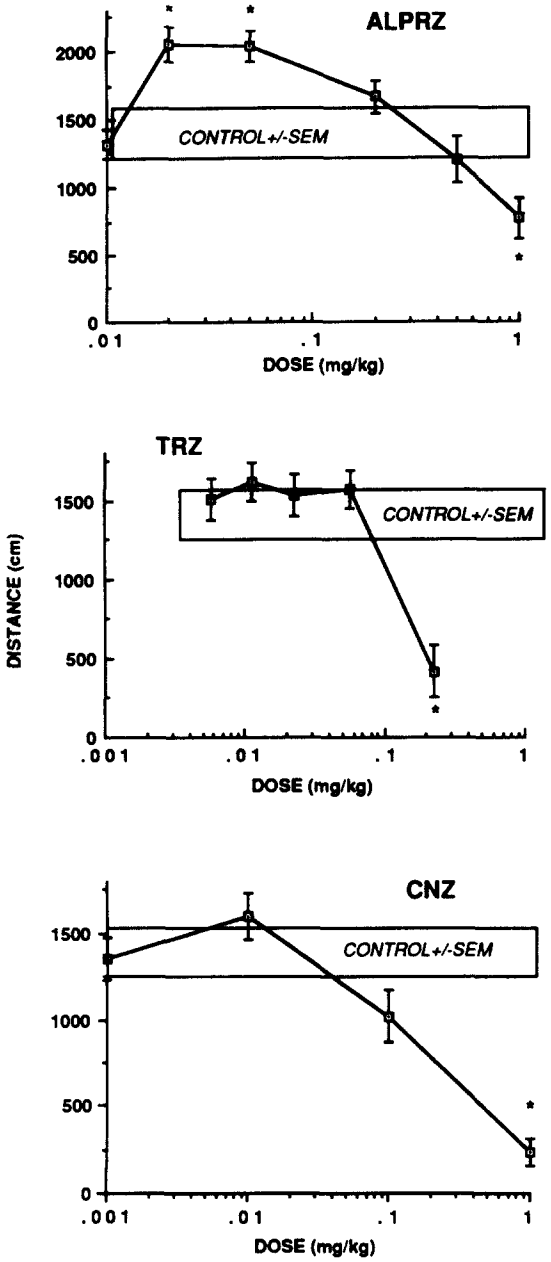

FIG. 1. Effects of alprazolam, triazolam and clonazepam on motor activity in mice. Mice were treated with varying doses of alprazolam (ALPRZ), trizaolam (TRZ), or clonazepam (CNZ) and a 5-minute open-field trial was performed after 1 hour. Results are distance traveled in cm during the trial, mean±SEM, n=10 at each dose and for controls. Enclosed areas represent mean±SEM for vehicle-treated controls. Asterisks denote $p<0.05$ vs. controls.

beams are spaced 2.65 cm apart across a Plexiglas enclosure 40 cm square. Interruption of each beam generates an electrical impulse which is processed by a microcomputer to record: distance traveled, ambulatory time, resting time, stereotypic time, stereotypic movements, vertical movements, and clockwise and counterclockwise rotations. Mice underwent a five-minute trial one hour after drug injection.

Data were analyzed using analysis of variance (ANOVA) and individual groups were compared using corrections for multiple comparisons.

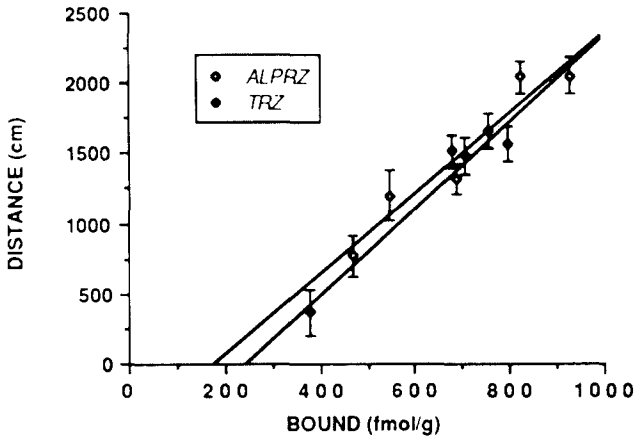

FIG. 2. Motor activity correlates with benzodiazepine receptor binding after acute treatment with alprazolam and triazolam. Data for distance traveled are from Fig. 1 above, and for receptor binding from [7]. Correlation coefficients are greater than 0.9 for alprazolam (ALPRZ) and triazolam (TRZ), and the regression lines are similar ($p<0.05$).

RESULTS

One hour after alprazolam administration, 1 mg/kg, mice displayed a decrease in distance traveled compared to vehicle-treated controls (Fig. 1). In addition, resting time was increased and ambulatory time, stereotypic time, and vertical movements were decreased (all $p<0.05$, data not shown). After a dose of 0.5 mg/kg, distance traveled and ambulatory time were slightly but not significantly decreased, while resting time was increased. All parameters were similar to controls following 0.2 mg/kg alprazolam. However, after a dose of 0.5 mg/kg, both distance traveled and ambulatory time were increased compared to controls, while other parameters were unchanged. Similar results were observed after a dose of 0.02 mg/kg. At the lowest dose evaluated, 0.01 mg/kg, all parameters were again similar to controls.

One hour after treatment with triazolam (0.2 mg/kg) distance traveled was markedly decreased (Fig. 1), while resting time was increased and ambulatory time, stereotypic time and vertical movements were decreased (data not shown). All measured parameters were similar to controls at the lower doses of triazolam evaluated, 0.05, 0.02, 0.01 and 0.005 mg/kg, except that vertical movements were decreased at 0.05 mg/kg.

After clonazepam administration (1 mg/kg), distance traveled and ambulatory time were markedly decreased, as were stereotypic time and vertical movements. Distance traveled and ambulatory time tended to be decreased after clonazepam (0.1 mg/kg) but these differences did not reach significance ($p<0.10$). All parameters were similar to controls at clonazepam doses of 0.01 and 0.001 mg/kg.

Distance traveled after alprazolam and triazolam were highly correlated with benzodiazepine receptor binding in vivo as determined in a prior study [7] (Fig. 2). Similar correlations were observed for ambulatory time, stereotypic time and vertical movements, and an inverse correlation was seen with resting time (data not shown).

Administration of a dose of the benzodiazepine antagonist Ro15-1788 (2 mg/kg) sufficient to fully occupy receptors ([10];

Miller *et al.*, submitted for publication) did not alter distance traveled (1673±128 cm), ambulatory time (61.2±4.8 sec) or stereotypic time or vertical movements (data not shown; all mean±SEM, n=10 in each group). However, concurrent treatment with Ro15-1788, 2 mg/kg, and alprazolam, 0.05 mg/kg, one hour prior to testing prevented the increase in distance traveled and ambulatory time noted above.

DISCUSSION

Alprazolam has been reported to have a unique clinical spectrum among benzodiazepines, based on clinical reports of efficacy in panic disorder and possibly depression [2,4]. Such effects in addition to the usual anxiolytic and hypnotic effects of benzodiazepines have not been reported for other triazolobenzodiazepines such as triazolam and estazolam [4]. Whether a neurochemical basis exists for the apparently unique spectrum of alprazolam is uncertain, although several reports have implicated interactions with adrenergic receptors or antagonism of platelet activating factor [6,13]. We have recently reported that alprazolam has unusual binding properties at the benzodiazepine receptor as determined by in vivo binding techniques [7]. These methods avoid limitations of tissue preparation and temperature and buffer conditions that are associated with in vitro brain membrane assays [9]. We have previously reported that one hour after administration of alprazolam, 0.02 or 0.05 mg/kg, corresponding to cortex concentrations of 2–8 ng/g, benzodiazepine binding was increased above control values. At higher doses, alprazolam administration resulted in a decrease in in vivo binding of [^{3}H]Ro15-1788 in a similar fashion to other benzodiazepines, and at lower doses binding was similar to controls. In contrast, no increase in binding was observed with the triazolobenzodiazepines triazolam or estazolam or with other benzodiazepines such as lorazepam or clonazepam [8].

Results from the present study provide a behavioral correlate for the unusual binding characteristics of alprazolam. At the same doses which resulted in increased binding [7], we found increased motor activity compared to control mice or to mice treated with higher or lower doses of alprazolam. Also in accordance with binding results, we found that motor activity was dose-related for triazolam and clonazepam [7,8], with no increase above controls across a broad dose range. That motor activity was linearly correlated with receptor binding for both alprazolam and triazolam suggests that this behavior is receptor-mediated, as previous studies with other benzodiazepines such as clonazepam have indicated [8]. Further, since this relation occurs for doses of alprazolam which augment activity, the unusual effects of alprazolam also appear to be receptor-mediated. Direct support for this hypothesis comes from the antagonism of low-dose alprazolam effects by a benzodiazepine antagonist, Ro15-1788.

Similar increases in benzodiazepine receptor binding and motor activity have been reported in stressed animals and in mice after benzodiazepine discontinuation [11]. It is possible that the behavioral and neurochemical responses observed with low doses of alprazolam are related to effects observed after discontinuation of this agent, or perhaps to its unique clinical spectrum. Evaluation of chronically-treated animals and of other receptor systems after low doses of alprazolam may further clarify the neurochemical basis for the effects of alprazolam.

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