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Central Nervous System Characterization of the New Cholecystokinin_B Antagonist LY288513

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HELTON, D. R., J. E. BERGER, J. F. CZACHURA, K. RASMUSSEN AND M. J. KALLMAN. *Central nervous system characterization of the new cholecystokinin_B antagonist LY288513*. PHARMACOL BIOCHEM BEHAV 53(3) 493–502, 1996. — The activity of LY288513, an investigational cholecystokinin (CCK)_B antagonist, was evaluated in a wide range of pharmacological tests in mice and rats. The anxiolytic benzodiazepine, diazepam, served as a reference standard for LY288513 in many of the tests. In the elevated plus-maze, LY288513 (3, 10 mg/kg, IP; 10, 30 mg/kg, PO) produced an anxiolytic-like action in mice with a magnitude of effect similar to that of diazepam. However, unlike diazepam, LY288513 produced no overt clinical signs and did not affect muscle tone, neuromuscular coordination, or sensorimotor reactivity. Also, in contrast to diazepam, LY288513 did not produce changes in the thresholds for electroshock- or pentylenetetrazol-induced convulsions. High doses of LY288513 (1000 mg/kg, PO) were required to reduce spontaneous activity levels, decrease body temperature, or potentiate the CNS-depressant effects of hexobarbital. LY288513 had no analgesic activity in mouse writhing or tail-flick tests. Electrophysiological studies in anesthetized rats showed that acute administration of LY288513 decreased the number of spontaneously active dopamine neurons in the substantia nigra and ventral tegmental area. However, LY288513 did not produce catalepsy. These data indicate that LY288513 possess both anxiolytic and antipsychotic potential.

Antipsychotic	Antianxiety	Behavior	CCK	Convulsions	Dopamine	Electrophysiology	LY262691
Schizophrenia	Substantia nigra	Ventral tegmentum					

CHOLECYSTOKININ (CCK) is a peptide that serves both as a gastrointestinal hormone and a neurotransmitter. Thus far, two types of receptors for CCK have been identified: CCK_A and CCK_B (17,23). Recently, antagonists selective for CCK receptor subtypes have been hypothesized to have therapeutic potential. For example, CCK_A antagonists may have utility for the treatment of pancreatitis and biliary pain (3,35), and CCK_B antagonists may have anxiolytic, antipsychotic, and analgesic potential (see 10,16, for reviews).

The therapeutic potential of CCK_B antagonists in anxiety-related disorders is supported by studies showing that CCK_B selective agonists (e.g., CCK₄ or pentagastrin) are anxiogenic in rats and mice (36). CCK_B agonists also induce panic attacks in normal controls and in patients with panic disorder (1,4,9). Further, CCK_B antagonists have anxiolytic activity in a variety of animal models of anxiety (2,27, Powell and Barrett, 1991, 31; Rataud et al., 1991, 36). As a result of these experimental

findings, CCK_B antagonists are currently under clinical investigation for the treatment of anxiety-related disorders. Currently, the most widely prescribed medications for anxiety disorders are the benzodiazepines. Although showing good efficacy in the treatment of anxiety disorders, benzodiazepines also produce significant adverse effects, including sedation, impaired motor function, dependence, and potentiation of other central-nervous-system (CNS) depressants (7,15,44). A new medication possessing anxiolytic activity without producing the side effect profile of the benzodiazepines would be a significant advance in the treatment of anxiety-related disorders.

CCK_B antagonists have also been hypothesized to have therapeutic potential for schizophrenic patients. The rationale for the use of CCK_B antagonists in schizophrenia builds on the hypothesis that hyperactivity of the brain's dopamine system plays a role in the etiology of schizophrenia (see 21, for re-

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view). Support for the dopaminergic-hyperactivity hypothesis of schizophrenia has come, in part, from electrophysiological studies showing that chronic, but not acute, administration of antipsychotic drugs decreases the number of spontaneously active dopamine neurons (5,6,42). This delayed disruption of dopamine neuronal activity parallels the delayed onset of therapeutic activity of the antipsychotic drugs in patients. More recently, CCK_B antagonists have been reported to inhibit the activity of midbrain dopamine neurons following acute administration (28,29). This acute effect of the CCK_B antagonists on midbrain dopamine neurons may be predictive of an antipsychotic action without a delayed onset.

CCK has also been postulated to play a role in the perception of pain. Several studies have suggested that CCK may function as an antagonist of endogenous opioids (13), and both CCK_A and CCK_B antagonists have been reported to enhance opiate analgesia (14,43). CCK_B antagonists have also been shown to prevent morphine tolerance (12,16). In addition, a CCK_B antagonist has been shown to have analgesic effects by itself in primates (25).

LY288513 is an orally active, selective CCK_B antagonist that is currently in clinical development for treatment of anxiety-related disorders. The racemate of LY288513 (LY262691) has been demonstrated to have anxiolytic properties in monkeys (2, Palmour et al., 1991) and to inhibit the activity of dopamine neurons in rats (28,29). However, a broad profiling of LY288513 for behavioral and electrophysiological effects has not been reported. In an effort to more fully evaluate the potential clinical utility of LY288513, we have examined its effects in a variety of behavioral and electrophysiological tests designed to explore some of its potential clinical indications and side effects.

METHODS

Animals

Male CD-1 mice (22–35 g) and male Sprague–Dawley rats (280–330 g) were obtained from Charles River Laboratories (Portage, MI), and acclimated at least 3 days before study onset. Mice and rats were housed at 23 ± 2°C (relative humidity 30% to 70%) and given Purina Certified Rodent Chow No. 5002 and water ad lib. The photoperiod was 12 h of light and 12 h of dark, with dark onset at approximately 1800 h.

Drugs

The following drugs were used: LY288513 [trans-N-(4-bromophenyl)-3-oxo-4, 5-diphenyl-1-pyrazolidinecarboxamide] (Eli Lilly and Company), Diazepam, pentylentetrazol (PTZ), and hexobarbital (Sigma Chemical Company, St. Louis, MO). For electrophysiological recording studies in rats, LY288513 was prepared in 50% ethanol. For all other testing in mice, LY288513 was suspended in 10% acacia in purified water. Diazepam was suspended in purified water by the dropwise addition of Tween 80. PTZ was dissolved in 0.9% saline. Hexobarbital was solubilized in purified water by the addition of 0.2 N sodium hydroxide. Hydrochloric acid (0.1 N) was subsequently added to adjust the pH prior to dilution to the final volume with purified water. Control animals received the respective vehicle. Food was removed at least 1 h before testing. In pilot studies using spontaneous activity, LY288513 (10–1000 mg/kg) produced maximal effects at 60 min postdosing. Therefore, 60 min was used as the primary time-point for evaluation of LY288513 in all behavioral experiments.

Elevated Plus-Maze

Construction of the elevated plus-maze was based on a design validated for mice by Lister (20). The maze was comprised of two open arms (30 × 5 × 0.25 cm) and two enclosed arms (30 × 5 × 15 cm). Corrugated paper was placed on the floor of each maze arm to provide texture. The arms extended from a central platform and angled at 90° from each other. The entire maze was made of clear Plexiglas. The maze was elevated to a height of 45 cm above the floor and illuminated by red light. Mice were individually placed on the central platform of the maze and the number of open and closed arm entries and percent time spent on various sections of the maze was recorded during a 5-min observation period. Arm entries were defined as entry of all four paws into an arm. Diazepam (0, 0.1, 0.3; IP) or LY288513 (0, 3, 10 mg/kg, IP; 10, 30 mg/kg PO) was administered 30 (based upon results of spontaneous activity testing where maximal effects were noted from 15 through 30 minutes) or 60 minutes before evaluation in the maze, respectively.

Clinical Observations

Clinical observations in mice were based on the method described by Irwin (18). Observations were made in color-coded animals by a blinded observer before (timepoint 0) and 60, 120, 360 min, and 24 h after administration of diazepam (0, 1, 3, 10 mg/kg, IP) or LY288513 (0, 100, 300, 1000 mg/kg, PO). Animals were observed for changes in autonomic function (respiration rate, lacrimation, salivation, urination/diarrhea, piloerection), awareness (hyperreactivity, hyporeactivity, aggressiveness, vocalization), motor activity (catalepsy, hypoactivity, hyperactivity, stereotype), and motor coordination/tone (hypotonic gait, ataxic gait, tremor). No effect (0) or effect (1) was recorded for each mouse observation. Individual scores were summed across all timepoints at each dose for each observation.

Spontaneous Activity

Activity of individual mice was recorded using Multi-Varimax Activity Monitors (Columbus Instruments, Columbus, OH). Interruptions of three individual infrared photocells were recorded by computer. Activity counts were cumulated after administration of diazepam (0, 0.1, 0.3, 1 mg/kg, IP) or LY288513 (0, 100, 300, 1000 mg/kg, PO) at 15-min intervals for 2 h. Ambulatory (locomotor or horizontal movement) counts represent the sum of individual photocell beam breaks ($n = 3$) within a representative time interval. Nonambulatory (stereotyped or nonlocomotor movement) counts represent the sum of the repetitive breaking of individual photocells.

Convulsive Models

Electroshock was conducted according to the method of Swinyard et al. (39), with the following modifications. Sixty minutes after administration of LY288513 (0, 100, 300, 1000 mg/kg, PO), saline was placed on each cornea immediately before placement of eyes in corneal electrodes. Current was then delivered with shock intensities of 6, 7, 8 or 9 mA (0.2-s shock duration). The types of convulsions (clonic, tonic, or tonic-extensor) observed within 10 s were recorded.

Four doses of PTZ (80, 90, 100, or 110 mg/kg, IP), were used to determine the effects of each dose of diazepam or LY288513. Diazepam (0, 0.3, 1, 3 mg/kg, IP) or LY288513 (0, 100, 300, 1000 mg/kg, PO) were given 30 or 60 min before

injection of PTZ. Immediately after dosing with PTZ, mice were placed into observation chambers and the types of convulsions (clonic, tonic, or tonic-extensor) observed across 10 min were recorded. This test is a modification of that described by Swinyard et al. (39).

Enzyme Competition/CNS Depression—Hexobarbital-Induced Sleep Times

Mice were given a single dose of diazepam (0, 1, 3, 10 mg/kg, PO) or LY288513 (0, 100, 300, 1000 mg/kg, PO) 60 min before challenge with hexobarbital (100 mg/kg, IP). Observation of the mice began immediately following hexobarbital injection. After the mouse was asleep, it was transferred to a prenumbered chamber and placed on its back. Mice were considered awake when they could successfully right themselves (all four feet in contact with the surface) or until 240 min had elapsed. If a mouse remained asleep for more than 240 min, then a latency of 241 was recorded. After a mouse righted itself, it was placed on its back once more and allowed to right a second time for confirmation. Sleeping times were recorded for each mouse.

Body Temperature

Rectal temperatures were recorded for each mouse before (timepoint 0), and 60, 120, 360 min, and 24 h after administration of LY288513 (0, 100, 300, 1000 mg/kg, PO). A Fluke (John Fluke Mfg. Inc.) thermometer probe was inserted in the rectum approximately 1 cm and body temperature was permitted to stabilize. After temperature stabilized, the measure was recorded within 0.1 degree C.

Neuromuscular Function—Grip Strength

The apparatus used was a modification of that described by Meyer et al. (22). The ability of each mouse to grasp each of two bars (forelimb or hindlimb) was examined. Values for this test are recorded as force in grams/trial. Each mouse was tested before (timepoint 0), and 30, 60, and 120 min after administration of diazepam (0, 1, 3, 10 mg/kg, IP) or before (timepoint 0), and 60, 120, and 360 min after administration of LY288513 (0, 100, 300, 1000 mg/kg, PO).

Neuromuscular Coordination—Rotarod

Mice were placed on a rotarod (model V EE/85, Columbus Instruments, Columbus, OH) accelerating from 1 to 80 revolutions/4 min. All mice were given 2 control trials at least 12 h before evaluation of LY288513. Mice were tested on the rotarod before (timepoint 0), and 30, 60, and 120 min after administration of diazepam (0, 1, 3, 10 mg/kg, IP) or before (timepoint 0), and 60, 120, and 360 min after administration of LY288513 (0, 100, 300, 1000 mg/kg, PO). The number of seconds each mouse remained on the rotarod was recorded.

Sensorimotor Reactivity—Auditory Startle

The auditory startle response of individual mice was recorded using San Diego Instruments startle chambers (San Diego, CA). Startle was assessed across 25 trials with a 5-min adaptation period at a background noise level of 70 ± 3 dBA, followed by 5 trial blocks of auditory stimuli (120 ± 2 dBA noise) presented at 8-s intervals. Baseline startle levels were established for each treatment group approximately 24 h before evaluation of LY288513. During testing, mice were given LY288513 (0, 100, 300, 1000 mg/kg, PO) 60 min before initiation of the startle trials.

Analgesic Activity

Sixty minutes following administration of LY288513 (0, 100, 300, 1000 mg/kg, PO), mice received 0.5% acetic acid (0.01 ml/g, IP). Mice were placed in individual clear observation chambers and the total number of writhes made by each mouse was counted between 5 and 10 min following the administration of acetic acid (34). Sixty minutes following dosing with LY288513 (0, 100, 300, 1000 mg/kg, PO), mice were placed in a holding tube and the time required for each mouse to react (tail flick) to the heat from a beam of light focused on the tail was recorded (Tail Flick Apparatus, Columbus Instruments Model 812, Columbus, OH).

Electrophysiological Recordings

Rats were anesthetized with chloral hydrate (400 mg/kg, IP); supplemental doses of anesthetic were administered through the lateral tail vein. The anesthetized rats were mounted in a stereotaxic apparatus (Kopf Instruments). While in the stereotaxic apparatus, body temperature was maintained at 35–37°C by placing the rat on a heating pad (K-module, American Pharmaseal Co.). After the rat's skull was exposed, a cisternal drain was performed to help prevent tissue swelling. Burr holes were then drilled in the rat's skull for placement of recording electrodes. To construct recording electrodes, single-barrel glass micropipettes (Radnoti, starbore glass) were pulled with a Narishige PE-2 vertical-puller; the resulting fine tips were broken back and the barrels back-filled with 2M NaCl. Electrode impedances were 1.8–2.6 M-ohm (in vivo measurements with a Dagan 2400 preamplifier utilizing a 2 Hz, 100 nA peak-to-peak square-wave). For recording neuronal activity, the band-pass filter on the preamplifier was set from 0.3 to 3 kHz. The tip of the recording electrode was lowered to the dorsal border of either A9 or A10, and then advanced using a micro positioning device (Burleigh, Inchworm Motor Controller), in 5- μ increments through the nucleus. The electrode was passed through 9 tracks (each track was separated by 0.2 mm), in a stereotaxically defined block of tissue (5.0–5.4 mm posterior and 2.0–2.4 mm lateral to bregma, and 6.0–8.5 mm ventral to the cortical surface for A9; 5.0–5.4 mm posterior and 0.5–0.9 mm lateral to bregma, and 6.0–8.5 mm ventral to the cortical surface for A10), and the number of spontaneously active dopamine cells was counted. A digital storage-oscilloscope (Gould 1604) was used to aid analysis of spike wave-forms; 8–16 wave-forms were captured and the averaged wave-form was displayed and analyzed. Cells were considered to be dopaminergic if they possessed the following characteristics: a long action-potential duration (> 2.5 ms); a triphasic wave-form with an initial positive phase usually containing a notch; and a slow, regular, or bursting firing-pattern with a rate of 2–9 Hz.

In preliminary findings LY288513 was determined to have its maximal effect on the number of spontaneously active dopamine neurons 2 h following IP administration; this interval from dosing to determination of drug effect was, therefore, employed for all electrophysiological tests. The number of spontaneously active dopamine cells was determined in 3 tracks, IP injections of LY288513 (0.1, 1, 10 mg/kg) or vehicle were made, and, 2 h later, the number of spontaneously active dopamine cells was determined in 6 additional tracks in the same animal.

Statistical Evaluations

Electrophysiological recordings were analyzed using an analysis of variance. Respective SI₅₀ values [shock intensity

(mA) inducing tonic-extensor convulsions in 50% of the mice for electroshock and CD_{50} values (doses producing tonic-extensor convulsions in 50% of the mice) for PTZ were calculated by probit analysis (log 10) and compared to respective SI_{50} or CD_{50} values obtained in the presence of LY288513 at the 95% confidence limit. Each treatment level for electroshock-induced or PTZ-induced convulsions was then analyzed using a frequency analysis. Elevated plus maze, hexobarbital-induced sleep time, acetic acid-induced writhing, and tail-flick were evaluated by an analysis of variance followed by a Tu-

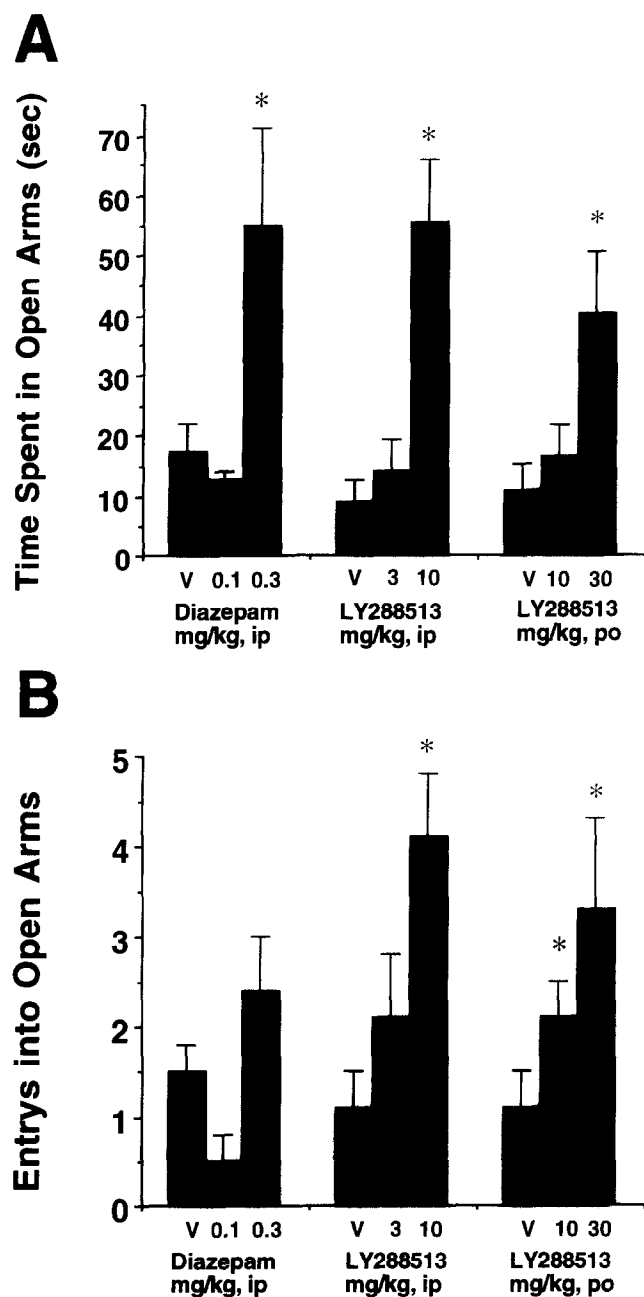


FIG. 1. The effect of diazepam (0, 0.1, or 0.3 mg/kg, IP; $n = 10$) or LY288513 (0, 3, or 10 mg/kg, IP; 0, 10, or 30 mg/kg, PO; $n = 10$) on the time spent on the open arms (A) and on the total number of arm entries (B) during a 5-min test on the plus-maze. Scores represent mean \pm SE. *Significantly different from control $p < 0.05$.

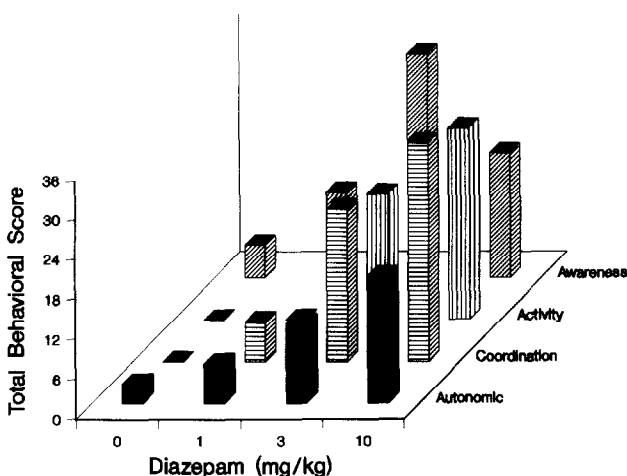


FIG. 2. Mean behavioral score of clinical observations occurring in mice receiving diazepam (0, 1, 3, or 10 mg/kg, IP; $n = 10$). Behavioral scores represent the incidence of observed behaviors totaled over all time points. *Significantly different from control $p < 0.05$.

key's standardized range test for post hoc comparison of group means. Spontaneous ambulatory and nonambulatory activity, grip strength, body temperature (difference from pre-dose), and rotarod were evaluated using an analysis of variance for repeated measures followed by Tukey's standardized range test for post hoc comparison of group means (33). Startle data were blocked into 5 consecutive 5-trial mean values. A mixed-design analysis of variance procedure was used for evaluating effects of dose and trial blocks, followed by a Tukey's standardized range test for individual post hoc group comparisons. Statistical analyses were performed using Statistical Analysis Systems (33). Effects with a probability of $p \leq .05$ were considered significant for the purpose of evaluating pharmacological activity.

RESULTS

Elevated Plus Maze

Diazepam administration (0.3 mg/kg, IP) produced significant (Tukey's HSD, $p < 0.05$) increases in time spent in the open arms (Fig. 1a), but not in total open arm entries (Fig. 1b). LY288513 administration produced significant (Tukey's HSD, $p < 0.05$) increases in both time spent in the open arms (10 mg/kg, IP; 30 mg/kg, PO) (Fig. 1a) and total open arm entries (10 mg/kg, IP; 10, 30 mg/kg, PO) (Fig. 1b). Although LY288513 was active in increasing arm entries and time spent in open arms by both the IP and oral routes, the compound was approximately 3 times less potent by the oral route.

Clinical Observations

Diazepam administration resulted in autonomic changes at doses of 1, 3, and 10 mg/kg with some mice exhibiting piloerection or slowed respiration. Biphasic changes in awareness were noted with hyperactivity observed at 1 and 3 mg/kg and hyporeactivity observed at 10 mg/kg. Deficits in motor coordination (hypotonic gait) were seen at 1, 3, and 10 mg/kg and mice showed hypoactivity at doses of 3 and 10 mg/kg (Fig. 2). The majority of observations with diazepam were noted at 60 and 120 min, with only hypoactivity remaining at 360 min. No overt observations were seen at 24 h postdosing.

LY288513 produced no notable clinical signs at any dose examined (data not shown). Additionally, LY288513 did not produce catalepsy.

Spontaneous Activity

Diazepam administration significantly reduced ambulatory (Fig. 3a) and nonambulatory (Fig. 4a) activity levels at 3 mg/kg from 15 through 30 min postdosing (Tukey's HSD, $p < 0.05$). A trend toward increased activity was noted at lower doses of diazepam; however, this increase was not significant. LY288513 administration reduced both ambulatory (Fig. 3b) and nonambulatory (Fig. 4b) activity levels at 1000 mg/kg from 45 to 60 min postdosing (Tukey's HSD, $p < 0.05$).

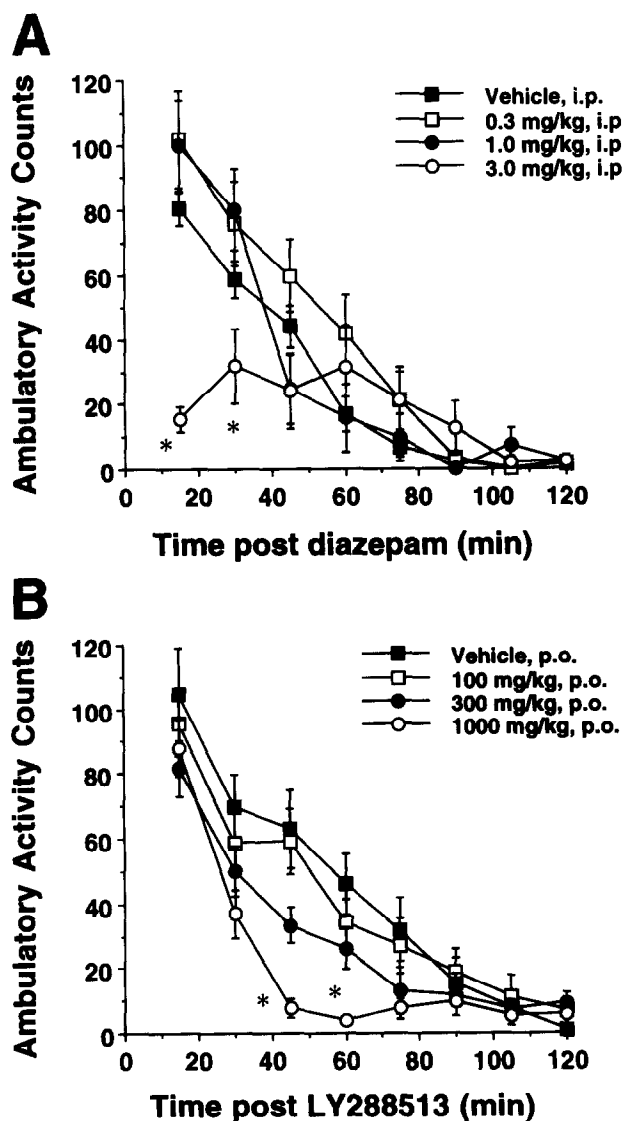


FIG. 3. Mean (\pm SE) spontaneous ambulatory activity counts in mice accumulated at 15-min intervals for 2 h following administration of diazepam (0, 0.3, 1, or 3 mg/kg, IP; $n = 10$) (A) or LY288513 (0, 100, 300, or 1000 mg/kg, PO; $n = 10$) (B). Ambulatory counts represent the sum of individual photocell beam breaks. *Significantly different from control $p < 0.05$.

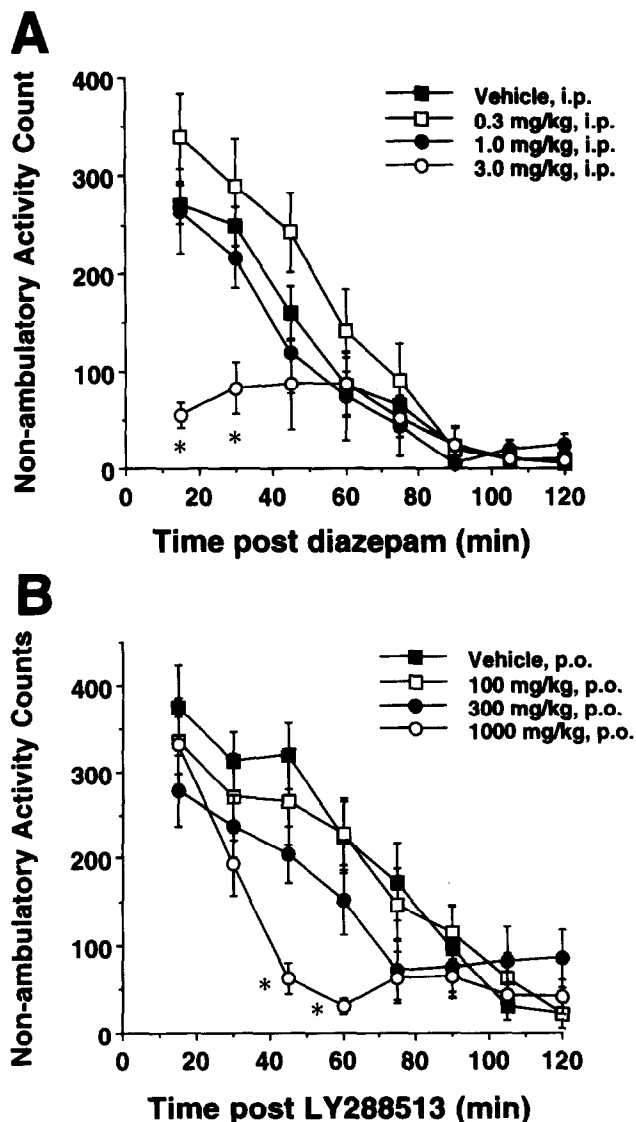


FIG. 4. Mean (\pm SE) spontaneous nonambulatory activity counts in mice accumulated at 15-min intervals for 2 h following administration of diazepam (0, 0.3, 1, or 3 mg/kg, IP; $n = 10$) (A) or LY288513 (0, 100, 300, or 1000 mg/kg, PO; $n = 10$) (B). Nonambulatory counts represent the sum of the repetitive breaking of individual photocells. *Significantly different from control $p < 0.05$.

Convulsive Models

Diazepam completely attenuated all PTZ-induced tonic convulsions at doses of 1 and 3 mg/kg (Table 1). No significant changes were observed in the frequency of PTZ- or electroshock-induced convulsive activity following administration of LY288513 (Table 1).

Enzyme Competition/CNS Depression— Hexobarbital-Induced Sleep Times

Diazepam produced dose-dependent increases in hexobarbital-induced sleep times with significant effects at 3 and 10 mg/kg (169% and 231% increases with respect to control, respectively) (Tukey's HSD, $p < 0.05$) (Fig. 5a). LY288513

TABLE 1
EFFECTS OF DIAZEPAM (IP) OR LY288513 (PO) ON PENTYLENETETRAZOL-INDUCED CONVULSION PATTERNS IN MALE CD-1 MICE (UPPER)

Percent Convulsions									
Diazepam (mg/kg)	PTZ (mg/kg)				LY288513 (mg/kg)	PTZ (mg/kg)			
	80	90	100	110		80	90	100	110
0	10	30	60	100	0	10	20	50	70
0.3	0	0	20	60	100	10	20	50	80
1	0	0	10*	0*	300	0	0	40	90
3	0	0	0*	0*	1000	10	40	70	80

LY288513† (mg/kg)	Current (mA)			
	6	7	8	9
0	20	50	50	100
100	10	40	40	70
300	0	30	60	100
1000	10	30	50	70

Effects of orally administered LY288513 on electroshock-induced convulsion patterns in male CD-1 mice (lower).

*Significantly different from control $p < 0.05$.

† $n = 10$ mice/treatment group.

produced dose-dependent increases in hexobarbital-induced sleep times with a significant (130% increase with respect to control) increase at 1000 mg/kg (Tukey's HSD, $p < 0.05$) (Fig. 5b).

Body Temperature

There were significant reductions in mouse body temperature at the high dose of 1000 mg/kg LY288513 (Tukey's HSD, $p < 0.05$) with a maximum decrease of 1.3°C from 60 through 120 min after dosing (Table 2).

Neuromuscular Function—Grip Strength

Reductions in both forelimb (30 through 60 min) and hindlimb (30 min) grip strength were seen following administration of diazepam (Table 3) with significant reductions at 10 mg/kg (Tukey's HSD, $p < 0.05$). No significant changes in neuromuscular function were noted after administration of LY288513 (Table 3).

Neuromuscular Coordination—Rotarod

Diazepam significantly reduced the number of seconds spent on the rotarod at 10 mg/kg 60 min postdosing (Tukey's HSD, $p < 0.05$) (Table 4). LY288513 administration did not produce any significant changes in neuromuscular coordination as evaluated on the rotarod (Table 4).

Sensorimotor Reactivity—Auditory Startle

There were no significant changes in sensorimotor reactivity, as evaluated using auditory startle responding, following administration of LY288513 (Table 5).

Analgesic Activity

LY288513 administration produced no significant changes in acetic acid-induced writhing or in the number of seconds to tail flick (Table 6).

TABLE 2
BODY TEMPERATURE CHANGES IN MALE CD-1 MICE ADMINISTERED LY288513 ORALLY

Time points (min)	Change in body temperature (°C)†			
	60	120	360	24 (h)
LY288513‡ (mg/kg)				
0	-0.4 ± 0.2	-0.6 ± 0.2	-1.4 ± 0.3	-1.1 ± 0.2
100	-0.5 ± 0.3	-0.6 ± 0.2	-1.2 ± 0.2	-1.1 ± 0.2
300	-0.6 ± 0.3	-0.7 ± 0.2	-1.2 ± 0.2	-0.8 ± 0.2
1000	-1.7 ± 0.3*	-1.9 ± 0.3*	-1.2 ± 0.3	-0.8 ± 0.3

*Significantly different from control $p < 0.05$.

†Difference from predose temperature, mean ± SE.

‡ $n = 10$ mice/treatment group.

TABLE 3
EFFECTS OF INTRAPERITONEALLY ADMINISTERED DIAZEPAM (UPPER) OR ORALLY ADMINISTERED LY288513 (LOWER)
ON FORELIMB AND HINDLIMB GRIP STRENGTH IN MALE CD-1 MICE

Time (min)	Grip Strength Force† (g)							
	0		60		120		360	
	Forelimb	Hindlimb	Forelimb	Hindlimb	Forelimb	Hindlimb	Forelimb	Hindlimb
Diazepam† (mg/kg)								
0	53.6 ± 5.4	21.2 ± 1.6	63.2 ± 4.1	23.5 ± 2.1	60.1 ± 5.0	25.1 ± 2.9	-	-
1	53.7 ± 4.6	24.6 ± 2.8	50.0 ± 3.9	26.6 ± 4.4	53.1 ± 3.4	26.0 ± 4.8	-	-
3	57.1 ± 6.5	21.1 ± 1.9	47.4 ± 5.0	20.4 ± 3.0	48.2 ± 3.6	20.4 ± 3.7	-	-
10	48.0 ± 5.1	23.8 ± 3.7	25.0 ± 3.4*	8.9 ± 2.4*	32.8 ± 4.9*	15.1 ± 3.1*	-	-
LY288513‡ (mg/kg)								
0	44.7 ± 3.3	14.4 ± 2.0	56.3 ± 3.7	15.8 ± 1.6	53.5 ± 2.8	13.1 ± 1.3	47.9 ± 3.8	15.0 ± 1.4
100	52.3 ± 4.6	15.5 ± 0.6	59.2 ± 3.7	17.8 ± 1.7	51.2 ± 3.9	16.0 ± 1.4	50.8 ± 2.7	16.4 ± 2.0
300	54.6 ± 2.1	14.7 ± 1.2	58.5 ± 3.2	14.5 ± 2.0	62.0 ± 3.5	16.9 ± 2.3	51.6 ± 2.9	15.9 ± 2.0
1000	48.0 ± 2.2	18.2 ± 1.5	53.4 ± 3.9	17.6 ± 2.0	55.1 ± 3.2	19.3 ± 2.4	46.3 ± 4.3	18.9 ± 2.2

*Significantly different from control $p < 0.05$.

†Values represent grip strength force (grams), mean ± SE.

‡ $n = 10$ mice/treatment group. </tbl>

Electrophysiological Recordings

Acute administration of LY288513 decreased the number of spontaneously active A9 and A10 dopamine neurons (Fig. 6).

DISCUSSION

In agreement with previously published reports (for review see 40), diazepam increased time spent in the open arms and number of open arm entries in the elevated plus-maze. The selective CCK_B antagonist LY288513 had similar effects on these parameters following both intraperitoneal and oral ad-

ministration (Fig. 1). These effects of LY288513 (a diphenylpyrazolidinone) confirm and extend previous reports demonstrating that CCK_B antagonists, from different structural classes (i.e., L-365,260, a benzodiazepine; and CI-998, a dipeptoid), display anxiolytic-like effects in the elevated plus-maze (16,36,37 Singh et al., 1991). These results also corroborate previously published reports indicating that the diphenylpyrazolidinone CCK_B antagonists LY262691 (the racemate of LY288513) and LY262684 have anxiolytic-like properties in primate punished responding tests (2).

In addition to anxiolytic activity, administration of diaze-

TABLE 4
EFFECTS OF INTRAPERITONEALLY ADMINISTERED DIAZEPAM (UPPER) OR
ORALLY ADMINISTERED LY288513 (LOWER) ON NEUROMUSCULAR COORDINATION
AS EVALUATED ON THE ROTAROD IN MALE CD-1 MICE

Time points (min)	Time on Rotarod† (s)			
	0	30	60	360
Diazepam‡ (mg/kg)				
0	50.9 ± 7.2	42.2 ± 7.8	60.1 ± 11.0	-
1	49.7 ± 6.9	55.3 ± 9.7	48.5 ± 11.4	-
3	43.7 ± 6.0	33.8 ± 6.2	51.3 ± 8.0	-
10	48.1 ± 3.7	31.9 ± 9.5	24.8 ± 4.9*	-
LY288513‡				
0	39.8 ± 7.6	52.7 ± 9.8	49.7 ± 8.7	53.8 ± 6.1
100	32.6 ± 5.4	33.2 ± 3.9	38.3 ± 8.6	42.5 ± 6.3
300	43.7 ± 7.1	40.7 ± 6.4	37.8 ± 5.5	45.8 ± 5.7
1000	32.3 ± 3.1	44.4 ± 7.7	34.7 ± 7.2	46.6 ± 13.6

*Significantly different from control $p < 0.05$.

†Values represent the mean (± SE) number of s mice remained on the rotarod.

‡ $n = 10$ mice/treatment group.

TABLE 5
EFFECTS OF ORALLY ADMINISTERED LY288513 ON AUDITORY STARTLE RESPONDING IN MALE CD-1 MICE

LY288513† (mg/kg)	Peak response (V_{max})† Trial block					Overall§
	1	2	3	4	5	
0	91.7 ± 10.0	74.5 ± 7.6	64.4 ± 6.2	53.9 ± 6.4	50.3 ± 5.7	67.0 ± 3.4
100	105.9 ± 11.5	64.3 ± 6.2	68.1 ± 7.7	54.8 ± 5.5	50.1 ± 6.1	68.7 ± 3.6
300	105.0 ± 10.3	87.2 ± 7.7	81.6 ± 7.2	62.7 ± 6.8	70.9 ± 7.4	81.5 ± 3.7
1000	91.6 ± 12.6	67.4 ± 10.0	64.3 ± 9.0	59.0 ± 9.1	64.9 ± 11.6	69.5 ± 4.7

†Values represent maximum auditory startle response (maximum voltage; V_{max}), mean ± SE.

‡ n = 10 mice/treatment group.

§Values represent maximum auditory startle response (maximum voltage; V_{max}), mean ± SE, collapsed over 25 trials.

pam and other benzodiazepines can result in sedative and ataxic side-effects (15, 7). In the present study, diazepam produced sedation at doses only 10-fold higher than those producing anxiolytic effects in the elevated plus-maze (Figs. 1,3,4). These same doses of diazepam also produced a dose-dependent reduction in neuromuscular function (Tables 3,4). However, LY288513 did not produce changes in neuromuscular function at any dose examined, and only produced sedation at doses 100-fold greater than those needed to have anxiolytic effects in the elevated plus-maze (Figs. 1,3,4; Tables 3,4). The differences in the side-effect profiles between diazepam and LY288513 are also reflected in the results of clinical observation tests. In these tests, diazepam, but not LY288513, administration resulted in marked changes in autonomic function, coordination, activity, and awareness.

Another side effect of benzodiazepines is the potential for interaction with other CNS depressants. Potentiation of hexobarbital-induced sleep time was used to evaluate potential interactions of diazepam or LY288513 with CNS depressants. Hexobarbital is a short-to-intermediate acting barbiturate that exerts its primary pharmacological effect on the CNS by enhancing inhibition of GABA-mediated neurotransmission (24). Test compounds may shorten or prolong hexobarbital-induced sleeping time by acting directly on the CNS (excitation or depression) or indirectly via inhibition of hepatic enzymes involved in hexobarbital metabolism. Diazepam produced a substantial dose-related interaction with hexobarbital with a 231% increase in sleep times at 10 mg/kg. However, hexobarbital-induced sleep times were increased by LY288513 (169%) only at the highest dose examined (1000 mg/kg) (Fig. 5b). These results indicate that, unlike the benzodiazepines, LY288513 may not produce CNS depression or interact with other CNS depressants at clinically relevant doses.

Diazepam is also used clinically as an anticonvulsant. Changes in ECS or PTZ convulsive thresholds following administration of LY288513 or diazepam were evaluated. Although diazepam increased the threshold for ECS- (unpublished data) and PTZ-induced convulsions, no significant changes in convulsive thresholds were seen with LY288513 (Table 1). These data suggest that LY288513 may not possess anticonvulsant activity in man.

The startle response elicited by external stimuli is mediated by a short-latency reflex through a well-defined neural circuit (8). Quantification of the startle response amplitude can be used to measure changes in sensorimotor reactivity. The startle response can also be used to examine sensitization or habituation to stimuli. Previously, doses of 3–30 mg/kg diazepam (sc) or 30–100 mg/kg (IP) LY288513 have been shown to have no effect on baseline startle responding in rats (30). In the

present study, startle amplitude in response to repeated auditory stimuli in mice was not altered by LY288513, indicating that LY288513 does not produce changes in sensorimotor responsiveness (Table 5).

CCK_B receptors have been postulated to play a role in the perception of pain (13,14,25,43). Acetic acid-induced writhing and tail flick latencies were used to evaluate the analgesic activity of LY288513. LY288513 did not produce any changes in writhing or tail flick responding, indicating that the pain thresholds evaluated by these models were not altered (Table 6). These findings agree with a previous study indicating that the CCK_B antagonist L-365,260 does not have antinociceptive actions in rodent models of pain (12). Whether or not LY288513 has analgesic effects in primates, as has been observed with the CCK_B antagonist L-365,260 (25), remains to be determined.

Like its racemate LY262691 (28,29, Rasmussen et al., 1993), acute administration of LY288513 produced a decrease in the number of spontaneously active A9 and A10 dopamine cells (Fig. 6). Previously, chronic administration (i.e., 2–3 weeks) of antipsychotic drugs has been shown to be required to decrease the number of spontaneously active midbrain dopamine cells. This delayed onset of effect on dopamine cell activity corresponds to the delayed therapeutic onset of the antipsychotic drugs in humans, and suggests that the decrease in dopamine cell

TABLE 6
EFFECTS OF ORALLY ADMINISTERED LY288513 ON ACETIC ACID-INDUCED WRITHING (UPPER) OR TAIL FLICK ANALGESIA (LOWER) IN MALE CD-1 MICE

LY288513† (mg/kg)	Mean Writhes‡	Standard error
0	9.0	1.0
100	7.7	1.6
300	8.2	1.4
1000	5.7	0.9
	Mean§ (s)	Standard error
0	4.6	0.3
100	4.6	0.2
300	4.7	0.2
1000	4.9	0.2

† n = 10 mice/treatment group.

‡Values represent number of writhes within a 5-min observation period.

§Values represent time to tail flick in seconds.

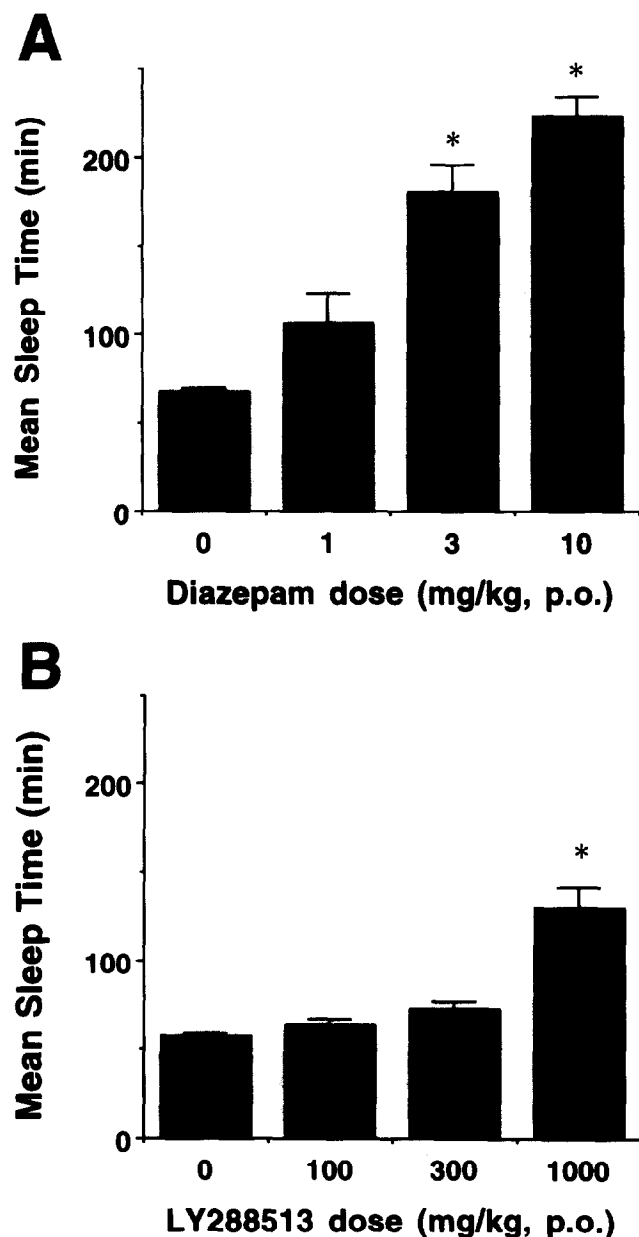


FIG. 5. Mean sleep times (min) in mice given diazepam (0, 1, 3, or 10 mg/kg, PO; $n = 10$) or LY288513 (0, 100, 300, or 1000 mg/kg, PO; $n = 10$) followed 60-min later by hexobarbital (100 mg/kg, IP). *Significantly different from control $p < 0.05$.

activity may be responsible for the therapeutic actions of antipsychotic drugs (6,42). Therefore, the acute effect of LY288513 on midbrain dopamine cells may be predictive of an antipsychotic action without a delayed onset. Additionally, currently prescribed antipsychotic drugs produce catalepsy. The production of catalepsy may be predictive of Parkinsonian motor side-effects in patients. Previously, it has been demonstrated that the administration of LY262691 does not produce catalepsy in rats (29, Rasmussen et al., 1993), and in the present study, clinical observations indicate that LY288513 does not produce catalepsy in mice. Therefore, LY288513 also may have a reduced propensity for producing extrapyramidal side effects. However, the interactions between CCK and dopamine are

complex, and recent studies have shown that the agonist activity at the CCK_B receptor can suppress various aspects of dopamine function, predicting antipsychotic potential for CCK_B agonists (for review see 41). Only detailed clinical investigations will be able to determine the antipsychotic potential of both CCK_B agonists and antagonists.

In addition to schizophrenia, activation of the A10 dopamine pathway may play a role in anxiety. The mesoprefrontal dopamine neurons originating in the A10 cell group have been shown to be activated by a variety of stressors (19, Kaneyuki et al., 1991; see 32, for review). Therefore, a decrease in the number of spontaneously active A10 cells may play a role in the anxiolytic activity observed for LY288513. It should be noted that the hypothesized role of the A10 system in anxiety and schizophrenia may be interrelated because stress has been hypothesized to play a role in the etiology of schizophrenic symptoms (11,38).

In conclusion, the selective CCK_B antagonist LY288513 has anxiolytic actions comparable to diazepam in the elevated plus maze. However, LY288513 does not possess the same profile of side-effects as diazepam. LY288513 did not impair neuromuscular function or sensorimotor responsiveness and did not produce adverse clinical observations at any dose examined. LY288513 potentiated the effects of a CNS depressant, slightly lowered body temperature, and had modest sedative effects only at the highest dose examined (1000 mg/kg, PO). LY288513 did not possess anticonvulsant or analgesic properties at any dose tested. In addition, acute administration of LY288513 decreased the number of spontaneously active A9 and A10 dopamine cells, but does not produce catalepsy. Therefore, LY288513 may be a novel anxiolytic and/or antipsychotic drug, with the potential for therapeutic effects in man without the side effects seen with currently prescribed benzodiazepines or antipsychotic drugs.

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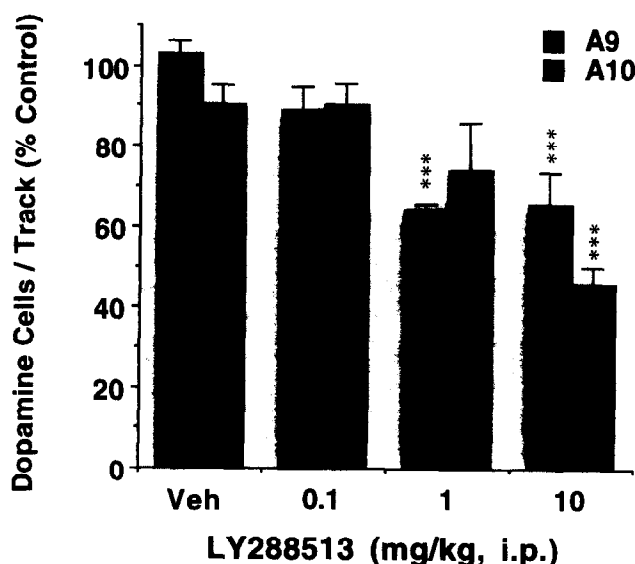


FIG. 6. Effects of acute administration of LY288513 on the number of spontaneously active A9 (solid) and A10 (hatched) dopamine cells; each point represents the mean \pm SE of 3–10 rats. ***Significantly different from control $p < 0.001$.

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