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Putative Benzodiazepine Partial Agonists Demonstrate Receptor Heterogeneity

SHU-WEN CHEN,[1] HENRY A. CHEN, M. FRANCES DAVIES AND GILDA H. LOEW

Molecular Research Institute, Palo Alto, CA 94304

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CHEN, S.-W., H. A. CHEN, M. F. DAVIES AND G. H. LOEW. *Putative benzodiazepine partial agonists demonstrate receptor heterogeneity.* PHARMACOL BIOCHEM BEHAV **53**(1) 87-97, 1996.—This study explored whether the behavioral heterogeneity of benzodiazepine receptor (BDZR) ligands is a consequence of multiple receptor subtypes or partial agonism. Putative partial agonists Ro16-6028, Ro23-1590, Ro23-0364, and abecarnil were compared with U78875, a mixed agonist-antagonist, and CGS8216, an inverse agonist, in five BDZR-mediated functions: hyperphagia, anxiolysis, sedation, hypothermia, and anticonvulsant activity. Only abecarnil was an agonist in all end points. Each of the other drugs exhibited qualitatively different responses at these end points. Specifically, Ro23-0364 produced no effect on body temperature, but was an agonist at other tests. Ro23-1590 had no effect on anxiolysis and hypothermia, but was an agonist at other tests. In contrast to other putative partial agonists, Ro16-6028 was found to be an antagonist in sedation and U78875 was an antagonist in hypothermia, but both were agonists at other end points. These qualitative differences in activity in the five behavioral end points studied cannot be explained by partial agonism at a single receptor and indicate that these ligands differentially activate multiple BDZR subtypes.

Ro16-6028 Ro23-1590 Ro23-0364 Abecarnil CGS8216 U78875 Hyperphagia Hypothermia Anxiolysis Locomotor activity Anticonvulsant Benzodiazepine Partial agonists

BENZODIAZEPINES receptor (BDZR) ligands are widely prescribed as medication for anxiety disorders, anesthesia, insomnia, and muscle relaxation. Although many BDZR ligands have proven clinical efficacy, the multiple effects elicited could include unwanted responses. For example, sedation or muscle relaxation may accompany a desired anxiolytic action. Thus, there has been increasing effort devoted to the development of BDZR ligands with selective in vivo activities. A major strategy used thus far by other investigators is based on the hypothesis of "partial agonism." This hypothesis assumes that the recognition of the same BDZR initiates multiple behavioral activities (12). According to the partial agonism theory (10-12), anxiolytic and anticonvulsant actions require less than complete receptor occupancy for a full response; sedation or muscle relaxation requires higher receptor occupancy (14). Thus, the generally accepted definition of a BDZR partial agonist is a compound that is a potent anxiolytic and anticonvulsant but that produces little or no sedation, hypnosis, or muscle relaxation (11,12). In this way, partial agonists may exhibit some degree of behavioral selectivity (24). However, receptor heterogeneity provides an alternative explanation for the selective actions of BDZR ligands. If different sets of receptor subtypes initiate different behavioral effects, then ligands that have some degree of receptor selectivity would have little or no in vivo effects mediated by those BDZRs for which they have low affinity, resulting in behavioral selectivity.

There is already substantial evidence for the existence of multiple $GABA_A$/BDZ receptors, from receptor binding studies and studies using molecular biologic techniques as well as from our own previous in vivo studies. Originally, type I and II receptors were distinguished by their high and low affinity to specific BDZR ligands (15). More recently, in our laboratory, we resolved three central BDZ binding sites in the rat spinal cord (18). Using photoaffinity labeling and autoradiographic techniques, $GABA_A$ receptor subunits were localized in various combinations in different brain regions and are believed to produce isoforms of the BDZRs (3). Expression of combinations of different $GABA_A$ receptor subunits in various transfected cell systems (17) produced functional BDZR

[1] Requests for reprints should be addressed to S.-W. Chen, Molecular Research Institute, 845 Page Mill Road, Palo Alto, CA 94304. Email: Shuwen@Purisina.Molres.Org.

with different rank orders of affinity of the same ligands, and varying ability of these BDZR ligands to activate them. From immunoprecipitation studies using subunit-specific antibodies, functional BDZRs with various binding profiles (21) and unique pharmacologic properties (20) have been identified. In a previous study (7), CGS9896, flunitrazepam, zolpidem, and AHR 11797 were examined in five behavioral tests. The behavioral heterogeneity exhibited by these compounds provided initial evidence for multiple functional BDZR subtypes and were not consistent with the single functional BDZR-based "partial agonism" theory (11,12).

In this study, we used a detailed comparison of the behavioral profile of six diverse ligands, including some putative partial agonists, assessed at the same multiple behavioral end points to distinguish further between these two possibilities. The objective of this study was to investigate the presence of behavioral heterogeneity of a given ligand, i.e., the ability of the same compound to have qualitatively different effects at different end points, for example, to be an agonist at one end point, an antagonist at another, and an inverse agonist at a third. The observation of such behavior would clearly distinguish between partial agonism and multiple functional BDZRs. Such behavior is not possible if there is only one functional BDZR. By contrast, the existence of multiple functional BDZRs can explain such an observation, as the ligand could have high affinity for a number of BDZR subtypes that initiate different behavioral responses and that have different requirements for activation.

To accomplish this goal, we assessed and compared four putative partial agonists, Ro16-6028 (bretazenil) (10,12), Ro23-0364 (19,23), Ro23-1590 (1,25), and abecarnil (24) with U78875, a compound that functions as a partial agonist in anxiolysis and sedation (27) but is an antagonist of hypothermia (26); and CGS8216, an inverse agonist in several behavioral end points (3). Figure 1 shows the structures of these diverse BDZR ligands.

The five BDZR mediated behavioral tests used were: anxiolysis in the elevated plus-maze, anti-pentylenetetrazol (PTZ)-induced convulsions, locomotor activity, palatable food intake, and change in body temperature. These end oints were found to be useful in our previous study (7) and should help in the further exploration of the validity of both the partial agonism and receptor heterogeniety hypotheses. They include two end points, anxiolysis and sedation, commonly used to define partial agonists (10,11,14), as well as a third, anticonvulsant activity, also used in the past for this purpose (1,14,19). There is also some evidence that body temperature is a useful test for discriminating between full agonists and partial agonists (13). The hyperphagic effects of most putative partial agonists have not been tested. These studies determined whether the definition of partial agonists were still valid when extending these studies to the examination of their hyperphagic and hypothermic effects. Therefore, the comparison among these five end points will be useful in discriminating between the partial agonism and receptor heterogeneity hypotheses of BDZR ligands.

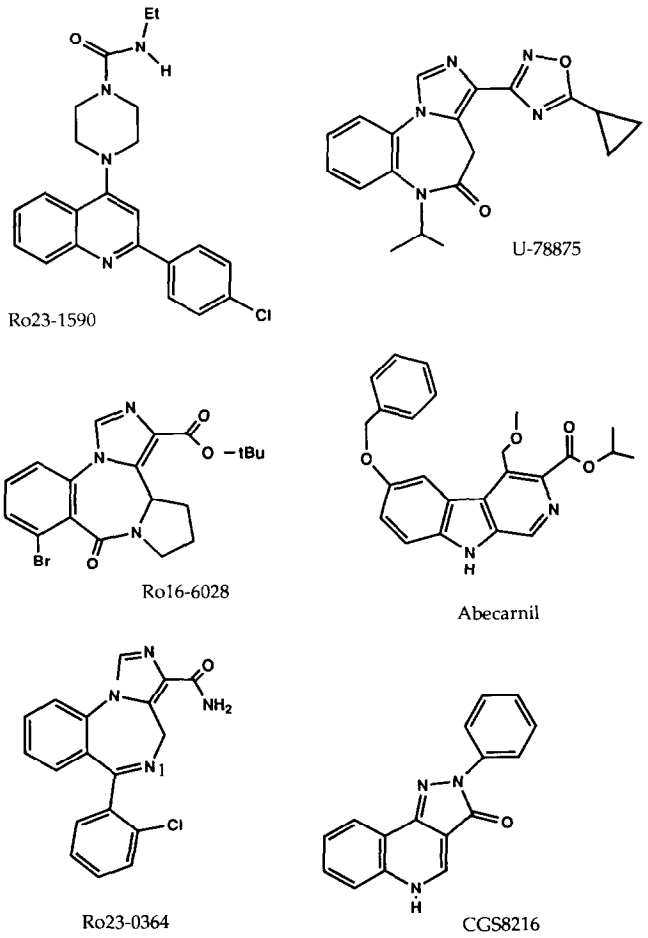

FIG. 1. The chemical structures of the six BDZR ligands.

METHODS

Animals

Male hooded Long Evans rats (Charles River, Wilmington, MA), weighing 300–450 g, were housed in pairs and maintained on a reversed 12 L : 12 D cycle for at least 2 weeks before initiation of behavioral testing. All tests were conducted during the dark phase of the light cycle, starting at 1100 h with at least a 1-h habituation period to the dimly illuminated test room before the administration of drug or vehicle and testing.

Drugs

The following compounds were received as gifts: Ro16-6028, Ro23-0364, Ro23-1590, Ro15-1788, and flunitrazepam from Hoffman-LaRoche (Nutley, NJ), abecarnil from Schering AG (Berlin, Germany), U78875 from Upjohn (Kalamazoo, MI), and CGS 8216 from Ciba-Geigy (Summit, NJ). Drugs were suspended in 40% (w/v) Encapsin (β-cyclodextrin) (American Maize, Hammond, IN), by sonicating for 20 min.

Behavioral Tests

Full dose–response curves with five to 10 animals per dose for all compounds in the five end points were generated. The dose range chosen was guided by previously published results of the same compounds (1,2,6,19,23,25,27,29,30). These behavioral tests were conducted using previously reported procedures (7): on 3 test days spaced at weekly intervals to eliminate the effects of prior drug administration. On the 1st test day, drug-induced changes of rectal temperature, performance in the plus-maze, and measurement of locomotor activity were sequentially measured. On the 2nd test day, food consumption

was measured. On the 3rd test day, proconvulsant/anticonvulsant activity was determined. The tests for antagonism were conducted using separate animals. The behavioral results were analyzed with one-way analysis of variance (ANOVA) using the Statview (BrainPower, Calabasas, CA) program; the treatment effect was separated using Dunnett's *t*-test. All drugs were given 30 min before the assessment of behavioral activity. In one case, because Ro23-1590 was reported to be slow-acting (1), the effect of Ro23-1590 on body temperature was monitored for 3 h. However, no differences were observed (data not shown). Characterization of antagonist activity was conducted only if the ligand displayed no agonist or inverse agonist activity. Flunitrazepam (5 mg/kg) was given 15 min before the administration of the ligand to be tested for antagonism, and the behavioral test was conducted 15 min after putative antagonist administration.

Hypothermia

Body temperature was recorded using a rat rectal probe (Digital Thermometer, Fisher Scientific, Pittsburgh, PA) before and 30 min after administration of the vehicle and test compounds. The first reading was taken to familiarize the animal with the experimental procedure and to ensure that any drug response was not masked by the small hyperthermic response caused by initial handling. To test the compound for antagonist activity, temperature readings were taken at 0, 15, 30, 45, and 60 min.

Anxiolysis/Anxiogenesis

A computer-controlled elevated plus-maze test system was adapted to study the anxiolytic and anxiogenic properties of the test compounds. The apparatus was mounted on a 50-cm-high plastic base and consisted of two open arms (50 × 10 cm) and two enclosed arms (50 × 40 × 10 cm) made from dark Plexiglas, connected by a central platform (10 × 10 cm). The apparatus was equipped with 12 pairs of infrared photocell units and connected to an IBM computer. Thirty minutes following drug or vehicle administration, the animal was placed in the center of the plus-maze, facing a closed arm. The number of entries and the time spent in the open, closed, and center arms were recorded over a 5-min period.

Sedation

The testing was done immediately after the anxiety test for a duration of 10 min. The locomotor activity monitor was an enclosed soundproof stainless-steel cubicle with a white Plexiglas bottom, 23 cm in diameter and 34 cm high, equipped with six pairs of photocell detectors. Interruptions of the photocell beams were recorded automatically by digital counter.

Food Consumption

The protocol for this test has been described and validated previously (4). Briefly, on the day before testing, rats were subjected to a sham experiment to allow habituation to the test diet and handling. The rats were deprived of food for 16 h, caged individually, and then given a preweighed cup of palatable modified rat chow pellets (diet 5729C-D; Purina Mill, BioServ, Frenchtown, NJ). On the test day, animals were injected with vehicle or drug 30 min before the introduction of the food cups. Food consumption was monitored for 1 h, after which time the food remaining in the cup was weighed and the amount consumed recorded.

Anticonvulsant/Proconvulsant Activity

To determine anticonvulsant activity, the BDZR ligands were administered intraperitoneally (IP) to rats 30 min before the IP injection of 60 mg/kg of PTZ. Rats were observed for 30 min, and the duration of convulsions and number of animals showing clonic seizures were recorded. The primary criterion used for anticonvulsant activity was the reduction of the duration of clonic convulsions. Only CGS8216, previously found to be a proconvulsant in mice (28), was tested for proconvulsant activity in this study. A subconvulsant dose of PTZ (20 mg/kg), IP, was given 30 min after the administration of CGS8216, and the number of rats undergoing clonic seizures and the duration of these convulsions were recorded.

RESULTS

Anxiolysis

As shown in Fig. 2, only three of the four presumed partial agonists, with the exception of Ro23-1590 [$F(4, 25) = 3.649$; $p = 0.0179$], were active at the anxiolytic end point in the elevated plus-maze test. CGS8216 [$F(7,42) = 6.799$; $p = 0.0001$] clearly displayed an anxiogenic profile. These results reinforce the ability of the plus-maze test to distinguish anxiogenic from anxiolytic activity, also found in our previous study (7). Ro16-6028 [$F(4, 47) = 19.42$; $p = 0.0001$], Ro23-0364 [$F(4, 45) = 2.593$; $p = 0.049$], abecarnil [$F(4, 21) = 6.57$; $p = 0.001$], and U78875 [$F(4, 44) = 3.188$; $p = 0.022$] significantly increased the time spent in the open arms. All of the anxiolytic ligands except Ro16-6028 also decreased the time spent in the closed arms. No effect of any of the six ligands was detected on the number of entries into the open or closed arms or the central platform (data not shown).

Sedation

When monitored in the locomotor activity apparatus, rats receiving these BDZR ligands were not completely ataxic. Two "partial agonists," abecarnil [$F(4, 21) = 9.178$; $p = 0.0002$] and Ro23-0364 [$F(5, 42) = 5.542$; $p = 0.001$], as well as CGS8216 [$F(7, 42) = 2.513$; $p = 0.03$] significantly reduced locomotor activity. U78875 [$F(4, 45) = 1.118$; $p = 0.14$; 5 mg/kg, $t = 2.78$, $p < 0.05$] and Ro23-1590 [$F(5, 42) = 1.78$; $p = 0.14$; 10 mg/kg, $t = 2.716$, $p < 0.05$; 20 mg/kg, $t = 2.765$, $p < 0.05$] displayed weak agonist activity in this test, whereas Ro16-6028 had no effect [$F(4, 45) = 1.35$; $p = 0.27$]. These results are all shown in Fig. 3.

Hypothermia

As shown in Fig. 4, two partial agonists, abecarnil [$F(4, 21) = 9.78$; $p = 0.0002$] and Ro16-6028 [$F(4, 47) = 11.05$; $p = 0.0001$], and CGS8216 [$F(7, 42) = 10.96$; $p = 0.0001$] decreased rectal temperature 30 min after administration of these ligands, whereas Ro23-0364 [$F(5, 42) = 0.424$; $p = 0.83$], Ro23-1590 [$F(5, 42) = 2.35$; $p = 0.058$], and U78875 [$F(4, 45) = 0.676$; $p = 0.61$] had no effect.

Hyperphagia

All of the BDZR ligands tested in this study showed activity in this end point. As shown in Fig. 5, five of them increased palatable food consumption after 16 h food deprivation: Ro23-1590 [$F(5, 42) = 2.78$; $p = 0.03$], Ro16-6028 [$F(4, 45) = 91.92$; $p = 0.0001$], Ro23-0364 [$F(4, 45) = 2.91$; $p = 0.01$], abecarnil [$F(5, 42) = 3.158$; $P = 0.01$], and U78875 [$F(5, 44) = 7.6$; $p = 0.0001$]. As reported previously (4),

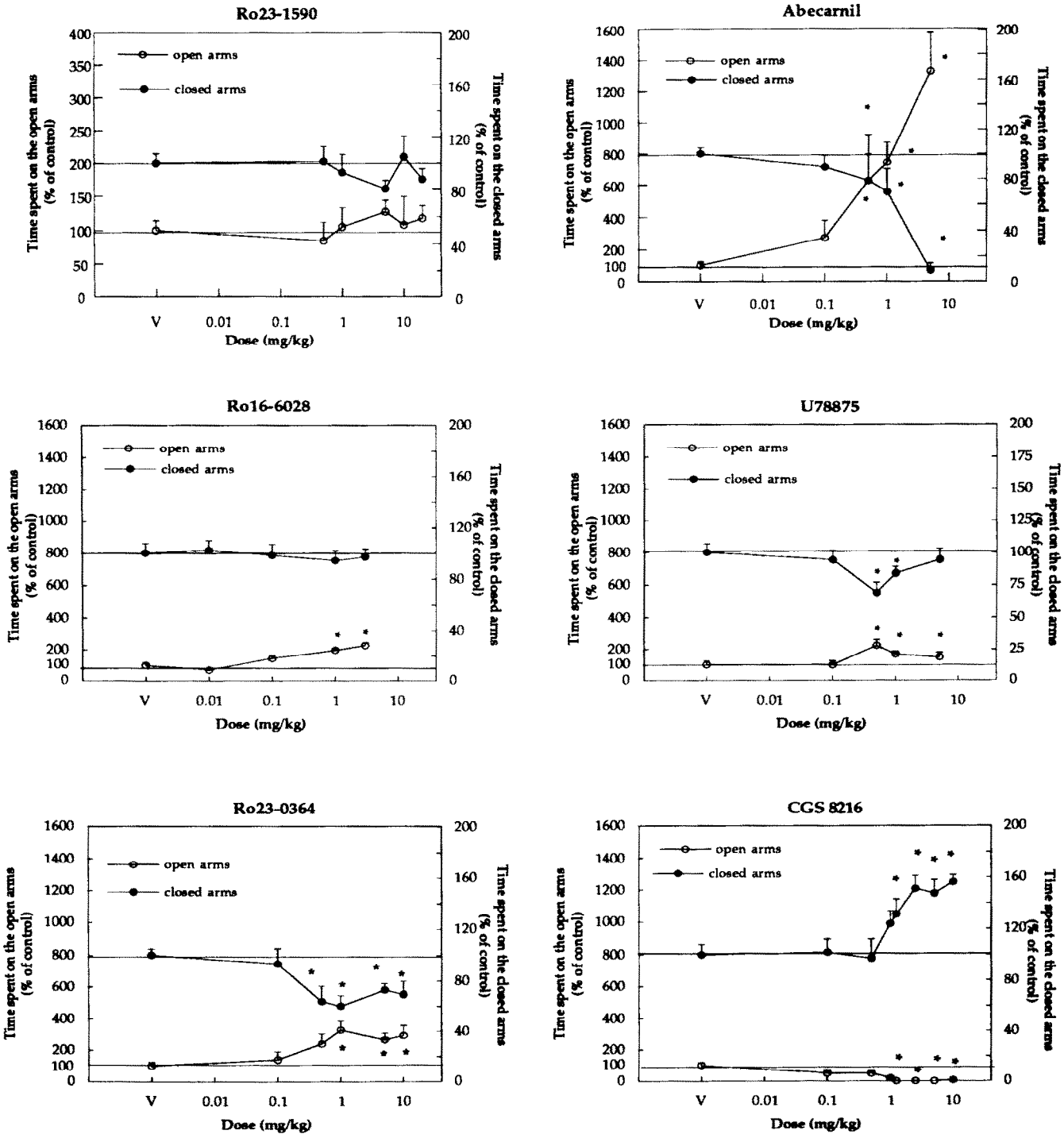

FIG. 2. The effect of six BDZR ligands on anxiolysis as tested in the plus-maze apparatus. For comparison among six compounds, data are presented as a percentage of control. See Methods for protocol. The parameter presented here is the mean ± SEM (n = 6–10). *$p < 0.05$, **$p < 0.01$, significantly different from vehicle-treated animals.

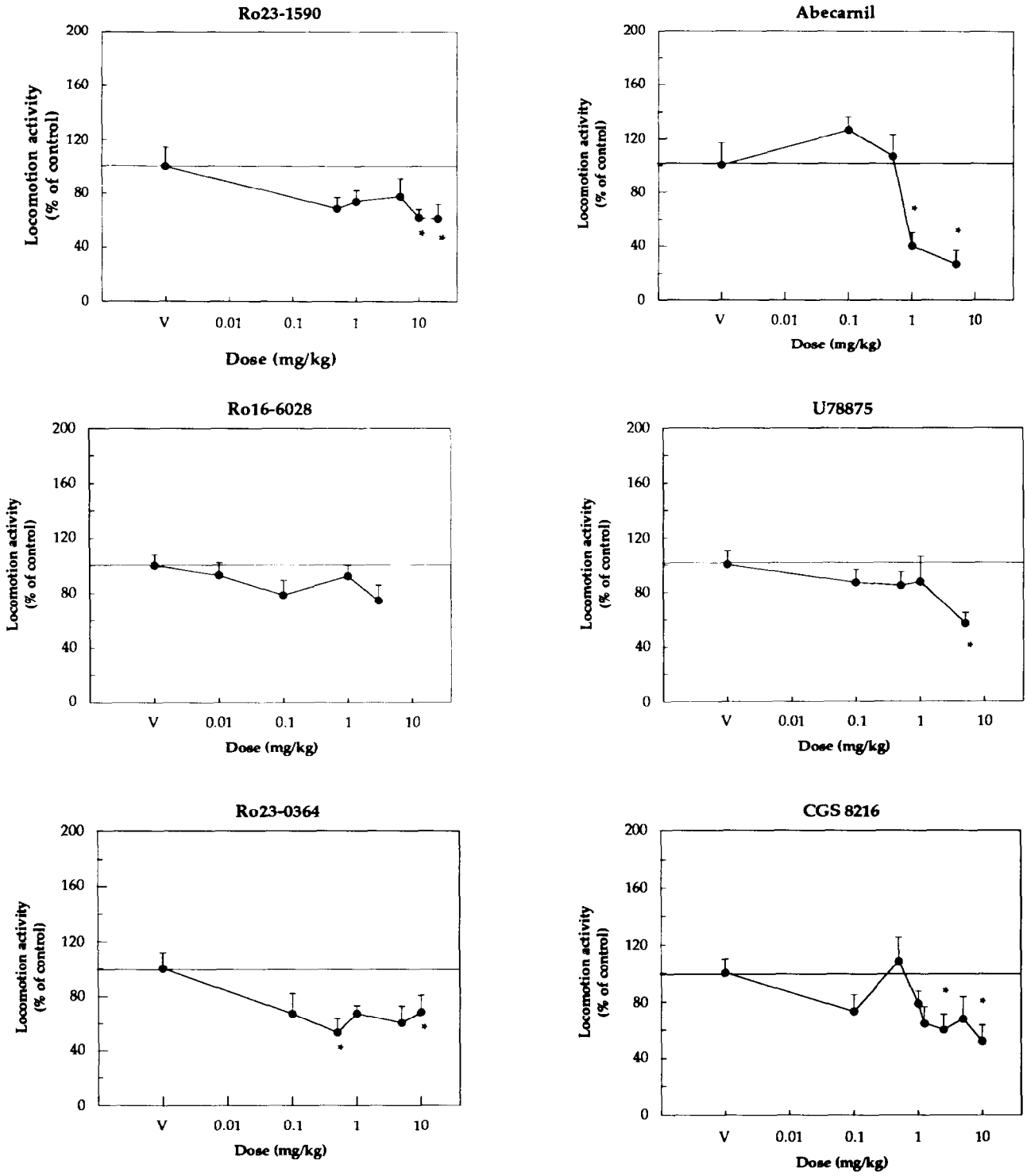

FIG. 3. The effect of six BDZR ligands on locomotor activity.

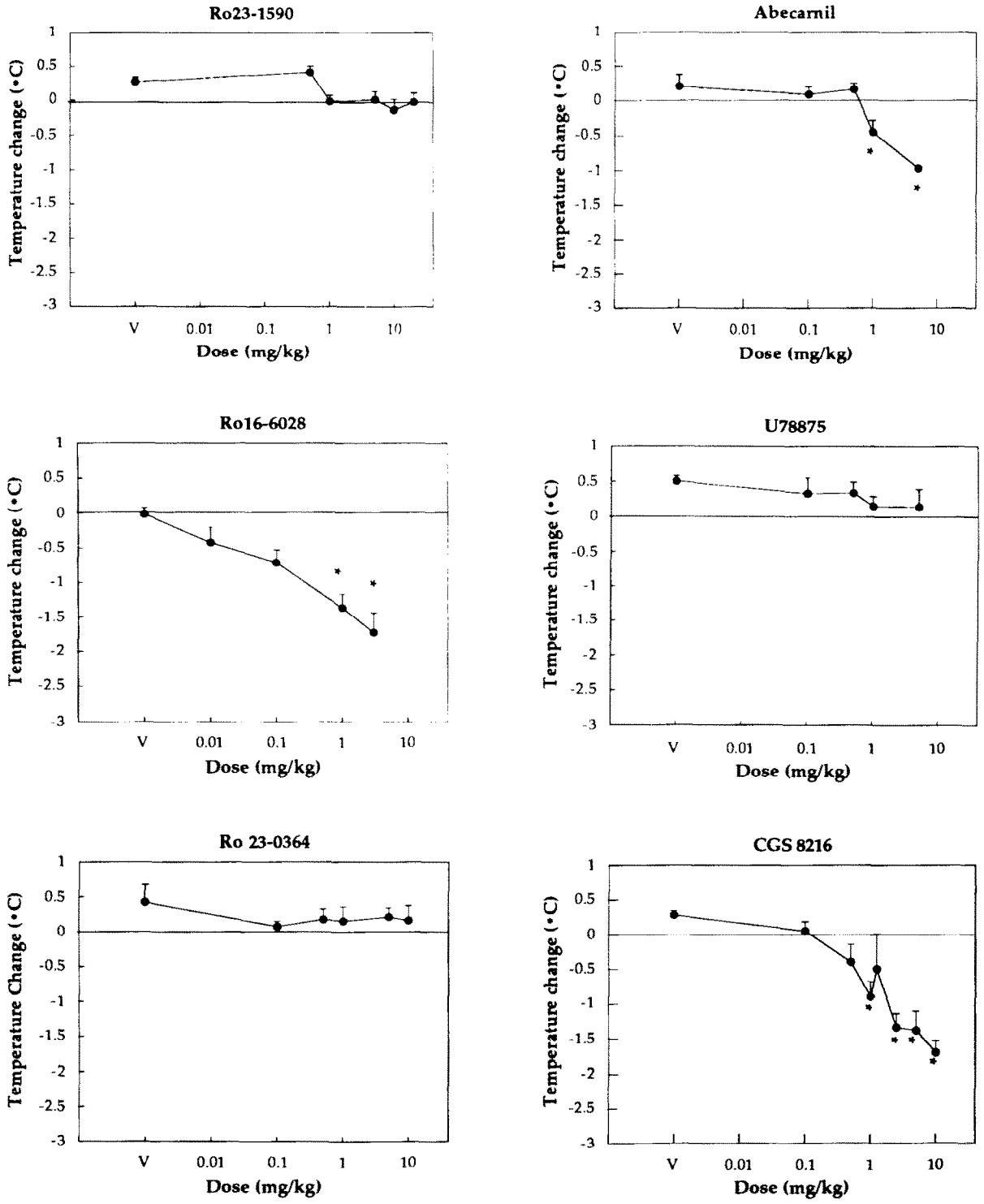

FIG. 4. The hypothermic effect of six BDZR ligands as measured by the change in rectal temperature before and after drug administration.

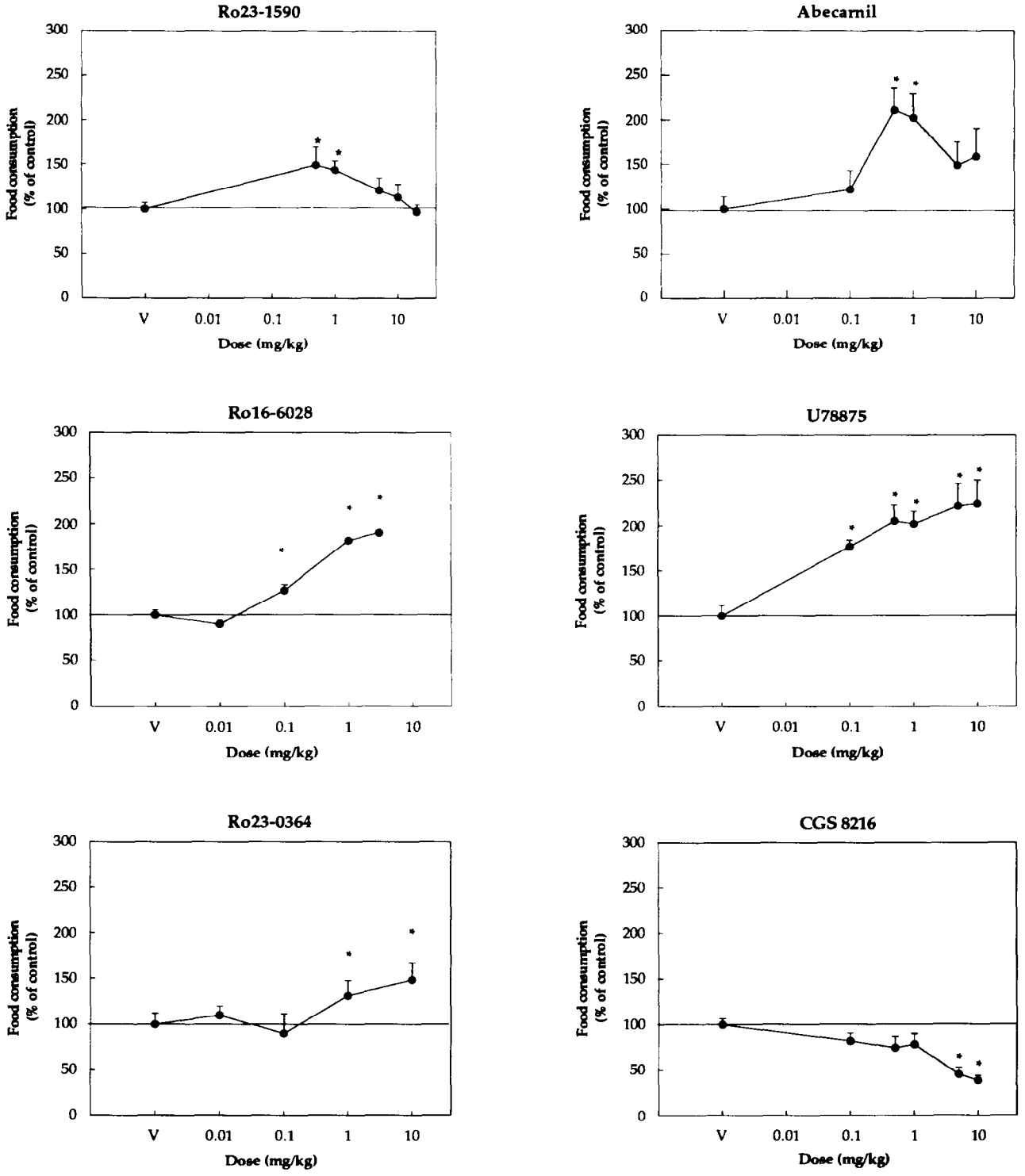

FIG. 5. Hyperphagic effect of six BDZR ligands as measured by palatable food intake in food-deprived rats. The result of CGS8216 has been previously reported (4).

CGS8216 decreased food consumption [$F(4, 44) = 6.167$; $p = 0.002$].

Anticonvulsant/Proconvulsant Activity

Figure 6 shows that five of the compounds tested were anticonvulsants, decreasing the duration of PTZ-induced clonic seizures: Ro23-1590 [$F(4, 22) = 4.783$; $p = 0.006$], Ro16-6028 [$F(5, 61) = 3.52$; $p = 0.0073$], Ro23-0364 [$F(4, 45) = 2.93$; $p = 0.03$], abecarnil [$F(5, 42) = 5.27$; $p = 0.0008$], and U78875 [$F(6, 29) = 17.6$; $p = 0.0001$]. Abecarnil and U78875 prevented PTZ-induced seizures at high doses (Fig. 6). CGS8216 dose-dependently increased the duration of seizures [$F(2, 9) = 5.77$; $p = 0.02$] and the percentage of rats convulsing.

Identification of Antagonists

Ligands devoid of agonist or inverse agonist activity in the anxiolytic, sedative, or hypothermic endpoints were tested for their ability to reverse the effects of flunitrazepam (5 mg/kg). The results are summarized in Table 1. Ro23-1590, inactive as an anxiolytic or in affecting body temperature, failed to show antagonism to these two effects. Specifically, 10 mg/kg of Ro23-1590 was unable to reverse the anxiolytic or hypothermic effect induced by flunitrazepam. Ro23-0364 (10 mg/kg) also did not reverse the hypothermic effect of flunitrazepam. Only U78875 (10 mg/kg) was able to antagonize the hypothermic effect of flunitrazepam. Ro16-6028 (3 mg/kg) significantly reversed the effect of flunitrazepam in the locomotor activity test.

The results of all these behavioral studies allow us to classify the effects of each compound at each end point into four qualitatively different categories of activity: agonist, inverse agonist, antagonist, and no effect. A summary of these results for the six compounds in the five behavioral tests is shown in Table 2.

DISCUSSION

Using internally consistent procedures allowing comparison among different compounds and end points, it is clear from each row in Table 2 that each compound studied, except abecarnil, had a qualitatively mixed activity profile, encompassing, in addition to agonist activity, either antagonist, inverse agonist, or no activity at some end point. This type of observation cannot be explained by partial agonist theory (10–12) in which it is proposed that BDZR ligands bind to a single functional receptor that is differentially activated to produce different in vivo end points. If only a single subtype of the receptor existed and mediated all the functions, a ligand could not be an agonist in one end point, an antagonist or inverse agonist at another end point, and have no effect in still other end points. Precisely this behavior was observed for all ligands except abecarnil. This type of behavioral heterogeneity among the compounds tested in this study provides robust evidence for the presence of multiple functional BDZRs.

A second result of these studies provides additional support for the presence of multiple functional BDZRs. Examining each column in Table 2 corresponding to the behavior of all compounds at a given end point, it is clear that only for one pair of end points—hyperphagia and anticonvulsant activity—did each of the six compounds have the same qualitative activity in both. Comparing each of the other nine possible pairs of in vivo behavioral results, at least one compound had a qualitatively different activity at the two end points. These disparities observed in nine pairwise comparisons of end-point behavior also cannot be explained by a single receptor hypothesis.

For example, comparing anxiolysis and sedation, two end points frequently compared to determine "partial agonist" behavior, not only was the rank order of agonist activity not preserved, but three of the six compounds had qualitatively different activities at the two end points. Ro23-1590 had no effect on the anxiolytic end point but was an agonist in sedation; Ro16-6028 was an agonist at the anxiolytic end point and an antagonist in sedation, and CGS 8216 was an inverse agonist in the anxiolytic end point and an agonist in sedation. Similarly, comparing anxiolysis with hyperphagia, our finding of no activity in the anxiolysis end point and agonist activity in hyperphagia for Ro 23-1590 lends further support to previous studies by Cooper (5) and by our own laboratory (7) of a separation between these two activities.

The extension of previous comparisons to include the putative partial agonists studied here at the hypothermia end point also fails to substantiate the partial agonist hypothesis, and reinforces the presence of multiple functional BDZRs. A previous report (13) compared the profound hypothermic effect of "full agonists" loprazolam and ZK91296 and the lack of effect of the "partial agonist" Ro17-1812, implying that this end point also allowed the discrimination between full and partial BDZR ligands. The effects of abecarnil, Ro16-6028, Ro23-0364, and Ro23-1590 on body temperature have not previously been reported. Despite some sedative effect, Ro23-0364 and Ro23-1590 did not change body temperature. On the other hand, Ro16-6028 decreased body temperature with an effective dose of <1 mg/kg, but was an antagonist of the sedative effect of flunitrazepam. Moreover, the agonists at the hypothermic end point found here, such as Ro16-6028 and abecarnil, have been previously characterized as partial agonists using the comparison between anxiolysis and other end points (9,10). Therefore, the characterization of partial agonist is inconsistent when extended to the hypothermia end point.

The most consistent explanation of the behavioral profiles obtained here is that different in vivo end points are initiated by binding of ligands to different subsets of BDZR subtypes, and that these subtypes have different requirements for activation. Lack of any type of activity, agonist, antagonist, or inverse agonist at a given end point can then be explained by the low affinity of that particular compound for the BDZR subtypes that mediated that end point. Agonist activity of a given compound at one end point and antagonist or inverse agonist activity at another can be explained by high affinity of the compound for the BDZR subtypes initiating the two activities but different ability to activate each receptor subtype.

Implicit in this hypothesis is the prediction that compounds such as Ro23-1590 and Ro23-0364 that have no activity at some end points would be more subtype-selective than the other four compounds that are either agonists, antagonists, or inverse agonists at all the end points, and hence would bind with high affinity to all the subtypes initiating these end points. If the relative affinities of these ligands to receptors in different brain regions could be mapped, the relationship between their ability to recognize a given BDZR subtype and their activity at these behavioral end points could be probed.

The results of this study of six compounds at five end points expand work already reported in the literature. Comparison of the present and previously reported results, when available, reveal many similarities but some differences. It is

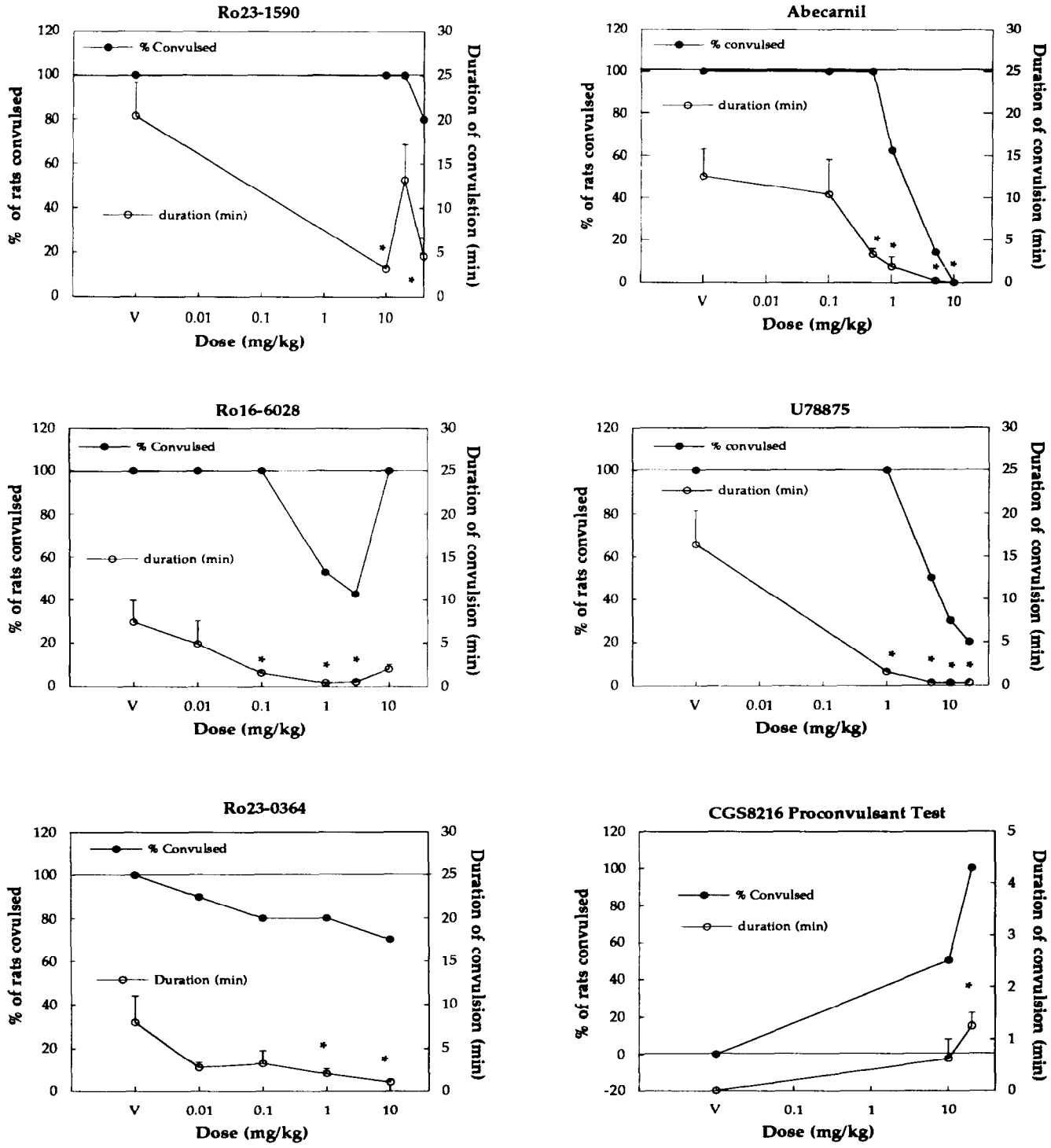

FIG. 6. Anticonvulsant action the BDZR ligands tested as the protection against PTZ (60 mg/kg)-induced clonic seizures. CGS8216 was tested as a proconvulsant after the administration of 20 mg/kg PTZ.

important for the inference of multiple BDZR subtypes that these discrepancies be reconciled.

One important disparity is that Ro23-1590 was not found to have an effect in the anxiolysis end point using the elevated plus-maze test in this study. In a previous study, robust dose-related antipunishment effect was observed in rats with the water-lick conflict test and in squirrel monkeys, using a single component lever-press conflict test (23). A possible reason for

TABLE 1

ABILITY OF PUTATIVE PARTIAL AGONISTS TO ANTAGONIZE 5 mg/kg OF FLUNITRAZEPAM

	Dose (mg/kg)	Anxiolysis Time Spent on Open Arms (s)	Sedation Locomotor Activity (Interrupts)	Hypothermia Temperature Change (°C) [Time (45)–Time (0)]
V + V			185.9 ± 12.6	
V + Ro16-6028	3		135.2 ± 42.5	
Flu + V	5		24.2 ± 7.0*	
Flu + Ro16-6028			119.6 ± 21.7†	
V + V				0.8 ± 0.37
V + Ro23-0364	10			1.3 ± 0.27
Flu + V	5			−1.48 ± 0.27*
Flu + Ro23-0364				−1.31 ± 0.31*
V + V		20.0 ± 11.6		0.01 ± 0.14
V + Ro23-1590	10	33.1 ± 13.3		0.05 ± 0.20
Flu + V	5	141.2 ± 49.3*		−1.28 ± 0.562*
Flu + Ro23-1590		185.7 ± 29.6*		−1.97 ± 0.194*
V + V				0.80 ± 0.27
V + U78875	10			0.25 ± 0.15
Flu + V	5			−1.48 ± 0.27*
Flu + U78875				−0.24 ± 0.20†

V + V: vehicle treated group; Flu + V: animals were given 5 mg/kg of Flu, then vehicle; V + drugs: animals were given vehicle then drugs; Flu + drugs: animal treated with both Flu and drugs. Values are expressed as mean ± SEM. The experiments were conducted using six to 10 rats in a group; data were analysed using one-way ANOVA. Dunnett's *t*-test was used to detect the treatment effect.

*Significantly different from V + V group.

†Significantly different from Flu + V group but no different from V + V group.

this disparity lies in our new observation that Ro23-1590 was more effective in inducing hyperphagia than anxiolysis (Figs. 1 and 5). Because the antipunishment tests can be confounded by drug-induced increased hunger or thirst (5), it is possible that the previous results were indicative of hyperphagic rather than anxiolytic behavior.

Another difference is that, although in previous studies Ro23-0364 (19), Ro23-1590 (23), and U78875 (26) were reported to have very little effect on sedative tests, we have found that all of these ligands significantly reduced locomotor activity even though not as robustly as flunitrazepam or zolpidem (7). The contradictions might lie in the species difference, the nature of tests used, and the routes of administration.

Although the presence of multiple functional BDZR subtypes is clearly implied from our behavioral studies, this hypothesis does not preclude the possibility that ligands could have a range of intrinsic activities (11,14,22) at a given BDZR subtypes. By direct measure of modulation of GABA-induced chloride flux in vitro (8,14), putative partial agonists such as Ro16-6028 and abecarnil have been shown to produce submaximal responses and require higher receptor occupancies when compared with diazepam or triazolam in rat cerebral cortex (8). This direct evidence for varying efficacy is totally compatible with the hypothesis of multiple BDZR subtypes. In fact, using molecular biology techniques, partial agonism and receptor heterogeneity have been found to coexist. In activation studies involving recombinant $GABA_A$ receptor subunits with varying a subunits and the same β- and γ-subunits, it was found that changes in the a subunit modulated the relative efficacy of BDZR ligands (16,22). For example,

TABLE 2

BENZODIAZEPINE RECEPTOR LIGANDS DEMONSTRATE FUNCTIONAL RECEPTOR HETEROGENEITY IN FIVE BEHAVIORAL TESTS

Ligands	Anxiolysis	Sedation	Hypothermia	Hyperphagia	Anticonvulsant Activity
Ro23-1590	No effect	Agonist	No effect	Agonist	Agonist
Ro16-6028	Agonist	Antagonist	Agonist	Agonist	Agonist
Ro23-0364	Agonist	Agonist	No effect	Agonist	Agonist
Abecarnil	Agonist	Agonist	Agonist	Agonist	Agonist
U78875	Agonist	Agonist	Antagonist	Agonist	Agonist
CGS8216	Inverse agonist	Agonist	Agonist	Inverse agonist	Inverse agonist

the combination of α_3-, β_2-, and γ_2-subunits (16) produced responses of Ro16-6028 and abecarnil equal to that of flunitrazepam on GABA-evoked chloride ion currents, but in the α_5-β_2-γ_2-receptor combination, abecarnil potentiated the GABA response to a lesser extent. These findings taken together demonstrate that BDZR ligands can be partial agonists in certain receptor subtypes but can be full agonists in other subtype of receptors. They reinforce the conclusion drawn from the behavioral studies made here, that the activities of BDZR ligands are mediated through different receptor subtypes.

ACKNOWLEDGEMENTS

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