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Novel discriminative stimulus effects of TPA023B, subtype-selective γ-aminobutyric-acid$_A$/benzodiazepine modulator: Comparisons with zolpidem, lorazepam, and TPA023

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ABSTRACT

Anxiolytics with fewer unwanted effects may be created by varying GABAergic efficacy at the BZ binding site across GABA$_A$ receptor subtypes. TPA023 and TPA023B have in vitro antagonist efficacy at α_1 subtypes and partial-agonist efficacy at $\alpha_{2/3}$ subtypes. TPA023B has partial-agonist efficacy at α_5; TPA023 has none. Drug discrimination procedures were used to determine whether the novel GABA$_A$ receptor efficacy profiles would be reflected in a model of subjective effects of BZ-site ligands. Rats were trained to discriminate TPA023, TPA023B, the nonselective BZ anxiolytic lorazepam, or the α_1-selective hypnotic zolpidem. The lorazepam, zolpidem, and TPA023 discriminations were learned in < 50 sessions. The TPA023B training group showed no evidence of acquiring the TPA023B discrimination after 160 sessions despite various procedural manipulations. Neither zolpidem- nor lorazepam-trained rats generalized to TPA023B. Within the same dose range, however, TPA023-trained rats generalized fully and dose-dependently to TPA023B. Number of training sessions to regain criterion discrimination performance following TPA023B tests in the lorazepam, zolpidem, and TPA023 groups increased as a function of dose, likely due to effects of residual TPA023B. Together with previous data, the present results suggest that elimination of α_1 efficacy plus reductions in $\alpha_{2/3}$ efficacy permits anxiolysis but decreases BZ-like interoceptive stimulus effects.

1,4-Benzodiazepines, such as diazepam and lorazepam, are effective, well-tolerated anxiolytics that represented a much safer alternative to barbiturates when they supplanted them for this clinical use in the 1960s (Lader, 1993; Ator, 2005). BZs also are useful as hypnotics and muscle relaxants, but these effects can be unwanted by outpatients treated for anxiety. A problem for chronic BZ use is physical dependence, which, coupled with nonmedical use and abuse, resulted in legal controls on BZ prescribing (Lader, 1993). Taken together these factors have decreased BZ use for long-term treatment of anxiety (Bond, 1993; Atack, 2003).

Discovery of the heterogeneity of BZ binding sites, and elucidation of the molecular biology and biochemistry of the GABA$_A$ receptor subtypes, raised the possibility that the multiple BZ effects could be mediated differentially as a function of GABA$_A$ receptor subtype, which suggested opportunities for the design and synthesis of BZ ligands with delimited sets of effects (e.g., Langer et al., 1990; Doble and Martin, 1992; Möhler et al., 2002). Native GABA$_A$ receptors that contain a BZ binding site are comprised of five subunits: two $\alpha_{1,\,2,\,3,\,\text{or}\,5}$, two $\beta_{1,\,2,\,\text{or}\,3}$, and one $\gamma_{1\ \text{or}\ 2\ (\text{short or long})}$ (Sieghart and Sperk, 2002). The composition of the BZ site, particularly the α subunit, long has been seen as critical to differential mediation of effects of BZ full agonists (Pritchett et al., 1989). An initial focus was on compounds that selectively bind α_1 subtypes under the premise that BZ-like abuse/dependence potential would be reduced (Klepner et al., 1979; Langer and Arbilla, 1988). Although this hypothesis was not borne out (Griffiths et al., 1992; Weerts et al., 1998; Ator, 2000, 2005; Ator et al., 2000), evidence for differential mediation of particular behavioral effects of BZs via GABA$_A$ subtypes has accumulated from experiments in transgenic mice and with nonselectively-binding BZ ligands that show differential efficacy in modulating GABA across GABA$_A$ subtypes (Rudolph et al., 1999; McKernan et al., 2000).

TPA023 (Atack et al., 2006) and TPA023B (compound 11; Russell et al., 2006) were synthesized in the context of a program to develop a BZ ligand that would be as efficacious and well tolerated as the BZs in treating anxiety but devoid of unwanted effects including abuse liability (Whiting, 2003; Dawson et al., 2005a). Both compounds bind BZ/GABA$_A$ subtypes nonselectively, but show no efficacy at α_1 subtypes. They are partial agonists in modulating GABA at $\alpha_{2/3/5}$ subtypes, and differ from each other

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in percentages of full efficacy at those subtypes (see Fig. 1). Their behavioral effects in rats are predictive of anxiolytic efficacy (Atack et al., 2006; Russell et al., 2006).

Drug discrimination data are important in establishing the subjective-effects profile of novel centrally acting compounds (Ator and Griffiths, 2003; cf., Goudie and Leathley, 1993). When TPA023 was studied in baboons trained to discriminate the nonselective 1,4-BZ anxiolytic lorazepam, it failed to show even partial lorazepam-like effects, which suggested that TPA023's subjective effects are unlike those of both BZ agonists and nonselective partial agonists (Ator, 2005). The purpose of the present study was to extend work with TPA023 but to focus on the discriminative stimulus effects of TPA023B to explore the hypothesis that its novel *in vivo* efficacy profile would make its subjective effects unlike those of a full agonist BZ ligand. TPA023B was used as a test drug in groups of rats trained to discriminate either (1) TPA023, (2) lorazepam, or (3) zolpidem, an imidazopyridine hypnotic that preferentially binds and is fully efficacious at the α_1 subtype, but also is efficacious at higher concentrations at $\alpha_{2/3}$ (but not α_5) subtypes. We also planned to train rats to discriminate TPA023B to be able to test the other three compounds in those animals. We predicted no generalization to TPA023B by lorazepam-trained rats given our previous findings with TPA023 and nonselective BZ partial agonists in lorazepam-trained animals (Ator, 2005). We predicted that lack of efficacy at α_1 subtypes would result in, at best, partial generalization in zolpidem-trained rats. Neither TPA023 nor TPA023B had been used as training drugs before the present study, but we predicted that rats would be trained to discriminate those two compounds using methods that previously had been successful with a range of compounds in rats. Because TPA023 has lower overall efficacy, we predicted it could be more difficult to train, especially given total lack of generalization to this compound in lorazepam-trained baboons. We predicted that if training could be accomplished, there would be full generalization to TPA023B in TPA023-trained rats and vice versa.

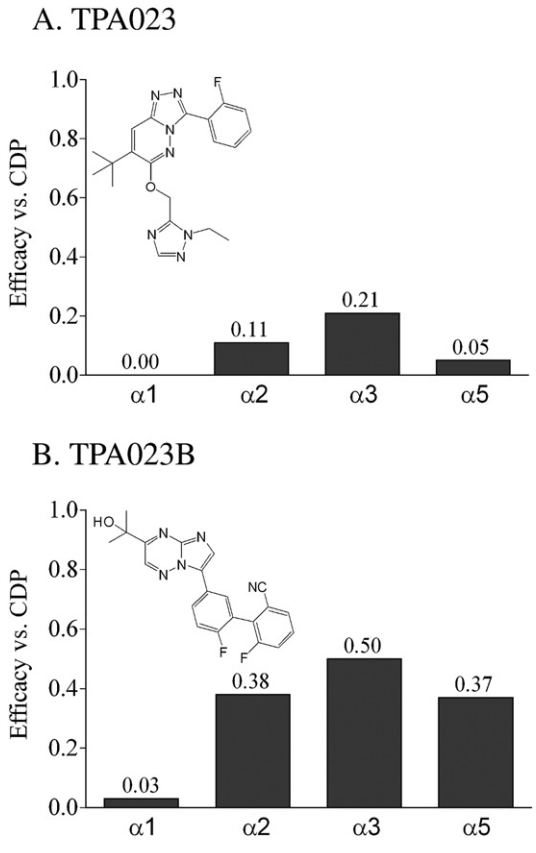

Fig. 1. Structures of TPA023 (Atack et al., 2006) and TPA0232B (compound 11, Russell et al., 2006) and their efficacies in modulation of GABA at cloned human $GABA_A$ receptor $\alpha_x\beta_3\gamma_2$ subtypes. Efficacy values are expressed relative to the nonselective full agonist chlordiazepoxide (CDP). Note: Human and rat affinity and efficacy data with BZ ligands tend to be comparable (Hadingham et al., 1993).

1. Methods

1.1. Subjects

Twenty-two male, Long–Evans Hooded rats (Harlan Sprague–Dawley, Blue Spruce Farms stock, Indianapolis, IN) were received in four groups of 5 or 6 rats each at 6weeks of age. They were individually housed in clear plastic cages (25.9 × 47.6 × 20.9cm) with an approximately 3cm depth of wood shavings, and had free access to food and water. Once training began, they were fed once per day approximately 30min following any experimental session. Weights were permitted gradually to increase during training, and were maintained at approximately 340 ± 10g. Room lights were on a 12:12 light/dark cycle (lights on at 6:00 a.m.). One or two non-nutritive rodent enrichment items (Bio-Serve, Inc., Frenchtown, NJ) were introduced to the home cage at various times with no change in behavioral performance during experimental sessions. Housing conditions and animal care and use were in accordance with U.S. Public Health Service Policy.

1.2. Apparatus

The six custom-made operant conditioning chambers are described elsewhere (Ator, 1991). Briefly, two rodent levers were mounted 13cm apart on one wall with identically colored cue lights mounted one over each lever that provided the only illumination. A food cup, into which 45-mg food pellets (P.J. Noyes, Lancaster, NH, USA) could be delivered, was centered on the chamber wall opposite the levers. The sound of an electromechanical relay operation accompanied each pellet delivery. White noise and a ventilation fan in a larger sound-attenuating enclosure masked extraneous sounds. Experimental events and the collection of behavioral data were controlled by an IBM-compatible computer, solid-state chamber interface cards, and MED-PC® State Notation software (Georgia, VT, USA). The computer program was custom-written for the laboratory, and included collection of data on a pellet-by-pellet basis. Lever presses and feeder operations also were monitored with an event recorder (Esterline-Angus, Indianapolis, IN, USA). Another clear Plexiglas operant chamber with an associated control console (Gerbrands Corp., Model 2150, Arlington, MA, USA) was used for initial training of the lever press for each rat by use of a separate, hand-operated switch that could operate the feeder. In that chamber, a cue light was over the lever, and the food cup was located next to the lever.

1.3. Drugs

TPA023 and TPA023B were generously donated by Merck, Sharp, & Dohme (Harlow, UK). Lorazepam was generously donated by Wyeth Research (Princeton, NJ, USA). Zolpidem hemitartrate was purchased from RBI (Natick, MA, USA). All drugs were given as i.p. injections in a volume of 1ml/kg, except that TPA023B at 18 and 32mg/kg were administered as 2ml/kg. TPA023B and TPA023 doses were dissolved in polyethylene glycol 400, which then was diluted 50% with 0.9% saline, and maintained for no more than 7days. Lorazepam doses were prepared as a stock solution of 80:20 propylene glycol:polyethylene glycol 400 (maintained for up to 30days), which then was diluted 50% with 0.9% saline (maintained for up to 7days) for injection. Zolpidem hemitartrate was dissolved in 0.9% saline in a volume of 20ml, and maintained until it had been used. Doses are expressed as the form of the drug given above (zolpidem base was 80.4% of the salt).

1.4. Drug discrimination training

Because of the importance of the outcome of training for the TPA023B group in relation to the other three, the methodology will be described in detail. The groups of rats were trained in the following order: zolpidem (n = 5), lorazepam (n = 6), TPA023 (n = 6), TPA023B (n = 5). Each rat first was habituated to the clear Plexiglas chamber and to the sound of the operation of the pellet feeder. Once pellets were being retrieved reliably when the feeder operated, lever press training by reinforcing successive approximations (shaping) began. Once the lever press was acquired, training switched to one of the six experimental chambers to which the rat was assigned.

Experimental sessions were conducted Monday through Friday with each group running within the same hour each morning. The cue lights over both levers were illuminated when the rat was placed in the chamber. Each rat was assigned a no-drug (ND) and drug (D) lever, the side positions of which were counterbalanced within each training group. Lever assignments also were counterbalanced across successive rats assigned to a particular experimental chamber. Shaping the lever press again was necessary due to the location of the food cup on the opposite wall, and shaping began on the ND lever. Pellet delivery was followed by a 1-s timeout, during which the cue lights were off and lever presses were counted, but had no programmed consequences. When a rat began to press the ND lever reliably, the response requirement was increased within and across the 20-min sessions until it was a fixed ratio (FR) of 5 responses per pellet. The criterion for raising the FR value by 1 was receipt of approximately 20 pellets (± 3) at a given FR value. Timeout was increased to 5s at FR 2 and to 10s at FR 5. A consecutive-response contingency was in effect such that pressing the other lever prior to completing the response requirement on the ND lever reset the count.

Drug discrimination training per se began when a rat reached FR 5 on the ND lever. Injections of zolpidem 2.0mg/kg, i.p., lorazepam 1.0mg/kg, i.p., TPA023 1.0mg/kg, i.p., or TPA023B 3.2mg/kg, i.p. occurred at pretreatment intervals (min) of 30, 60, 15, and 15, respectively. These intervals and doses were based on successful previous training for zolpidem and lorazepam in our laboratory, on evidence for discriminative effects of TPA023 in rats trained to discriminate flunitrazepam (Ator, unpublished), and on information that parameters for behavioral effects of TPA023B should be similar to TPA023 (Gerard R. Dawson, personal communication). Rats were placed in the experimental chamber for the last (or total) 15min of the pretreatment interval during which timeout was in effect. At the end of the presession timeout, the cue lights were illuminated and pellet reinforcement depended on meeting the FR contingency on the D lever. Initially, the response requirement was 1, with a timeout of 1s after each pellet; and shaping again was used to train the rat to press the D lever. After a rat was pressing the D lever, a double-alternation sequence of D and ND training sessions began (i.e., DD NDND DD NDND), all sessions were preceded by a 15-min timeout, and the timeout after each pellet delivery was 10s. The FR requirement was raised to 10 across sessions, generally according to the following sequence: D FR1–2, D FR 3–4, ND FR 3–4, ND FR 4–5, D FR 4–5, D FR 5–6, ND FR 5–6, ND FR 6–7, etc., but it was customized for individual rats to promote each rat's completing the sequence quickly and obtaining at least 20–25 pellets (usually more) per session under both D and ND conditions. The within-session increase on D training days occurred after at least 20 pellets had been obtained. Single alternation of D and ND training sessions generally began after the first FR10 session and performance was assessed to determine whether the performance criterion (see below) to be able to begin testing was met.

1.4.1. TPA023B training

After a mean of 11.8 alternating D and ND sessions at FR 10 at the 3.2mg/kg training dose, during which no rat met the criterion for testing, training conditions were individualized based on performance. Rats were given 4 to 6 sessions under any one condition before the next manipulation was made. Initially the approach was to raise the FR to values greater than 10 by 3 or 5 responses all at once, given that the number of pellets per session was > 30 for the previous four sessions. It was lowered on an individual basis if a rat failed to obtain at least 20 pellets in a session, and then gradually raised again to at least FR 10. When this manipulation was not proving successful at achieving criterion performance, the pretreatment time was increased to 30min. Next, the pretreatment time was held constant, but the dose was increased to 10mg/kg, and a double-alternation sequence of D and ND sessions was used. After 19 sessions, the FR was increased to 15 for an additional 16 sessions, this time with single alternation of D and ND sessions. The dose then was raised to 18mg/kg, and the pretreatment time was increased to 120min based on newly obtained pharmacokinetic information for rats (John R. Atack, personal communication). After 20 sessions, the FR was lowered to 10 for 10 additional sessions before increasing it to 15 for 19 sessions. In a final manipulation, which took the long elimination half-life of TPA023B into account, rats were given one day off (O) after a drug session (i.e., one cycle of training consisted of D O ND). This 3-session cycle was repeated for 13 cycles with no trend toward improvement in accuracy.

1.5. Generalization and substitution tests

The performance criterion was that (a) at least 95% of all responses in a session had to occur on the appropriate lever and (b) the first 10 consecutive lever presses had to be on the appropriate lever. Once this dual criterion was met in four consecutive sessions, a test session with the training drug vehicle and one with the training drug dose were given. Contingencies in test sessions were the same as training sessions except that making 10 consecutive responses on either lever produced a food pellet. These performances were assessed for demonstration of stimulus control by the training conditions. Stimulus control was demonstrated if (1) at least 80% of the total responses in the session were made on the ND lever in the session when vehicle had been injected, (2) at least 80% of the total responses in the session were made on the D lever in the session when the training dose had been injected, and (3) these results occurred in both test sessions sequentially. If the first and second elements of the stimulus-control criterion were not met, at least four D and ND single-alternation training sessions had to meet the performance criterion before both stimulus control tests were repeated.

Once stimulus control was demonstrated for zolpidem, lorazepam, and TPA023, tests could occur every third session, given that the performance criterion was met in the intervening ND and D sessions. Failure to meet criterion in any one training session required that criterion performance be shown in 4 consecutive training sessions in which D and ND alternated before the next test could occur. Dose–effect determinations were made for each group first with its training drug. Before testing with TPA023B, the zolpidem-trained rats were tested with CP730,330 (a partial BZ agonist), L-838,417, and dextofisopam; the lorazepam-trained rats were tested with CP730,330, dextofisopam, and pentobarbital; these data are not presented here. Tests with TPA023B were conducted with a pretreatment interval of 15min.

1.6. Assessment of the ataxic effects of TPA023B

Ataxia after TPA023B was assessed in the TPA023B group approximately 5months after the termination of training, during which the rats were not used in any experiments. The decision to make this assessment was based on a noticeable flaccid body tone after the first administration of 18mg/kg with a pretreatment of 120min. The procedure used was described by Melnick et al. (2002). Briefly, the frequency of "paw slips," defined as one paw falling below the floor rods in the experimental chamber and exposing the ankle

joint to the observer, was assessed in 5-min sessions for each rat. A baseline measurement of paw slips was made for each rat 120-min after a vehicle injection. Twenty-four hours later, rats were pretreated with 32mg/kg TPA023B 120min prior to a second 5-min determination of paw slips.

1.7. Data analysis

One-way analysis of variance (ANOVA) was used to determine whether the total number of sessions to meet the performance criterion for initial testing differed among the lorazepam, zolpidem, and TPA023 groups. For drug discrimination test sessions, responding across levers was calculated as the percentage of total responding on the D lever, excluding responses during timeouts. Consistent with the drug discrimination literature, full generalization was concluded if the percentage of D-lever responding was 80% or more. Conversely, 20% or less of D-lever responding was not considered to indicate D-like discriminative stimulus effects. Given the 95% accuracy criterion for training session performance, these thresholds can be considered significantly different from chance for a conditional discrimination (Sidman, 1980). Response rates were calculated by dividing the total responses on both levers when the cue lights were illuminated by the total session time minus timeouts. ED_{50} values were determined by drawing a line from the *Y*-axis at 50% to the group mean curve and from that point to the *X*-axis. ED_{50} values are reported to the nearest 1/8 $\log_{10}$-unit dose where the line met the *Y*-axis. The ED_{50} for reduction in rate of responding was determined in an analogous manner by using the mean rate of responding under vehicle test conditions as 100%, and dividing that rate by 2 to determine 50%. The number of sessions to meet criterion after novel doses of the training drug was analyzed separately for each group using a repeated measures ANOVA with the within-subjects factor of Dose (the lowest dose of TPA023 was omitted so that the number of doses would be equal across groups for this analysis). Sessions to meet criterion after novel TPA023B tests in zolpidem-, lorazepam-, and TPA023-trained rats were analyzed using a 3-×-5 mixed ANOVA with the between-subjects variable of Training Drug (lorazepam, zolpidem, TPA023) and the within-subjects variable of TPA023B Dose (0.32, 1.0, 3.2, 10, 32mg/kg) tested. Ataxia data are expressed as the mean (± SEM) number of paw slips under both conditions. A paired-samples *t*-test was used to compare paw slips after vehicle with those after TPA023B. All determinations of statistical significance were made at $p < 0.05$. Statistical analyses were conducted using the Statistical Package for the Social Sciences, Version 13.0.

2. Results

2.1. Sessions to criterion

The number of sessions until rats met the training performance criterion was counted from the session in which the D and ND training sessions began alternating (Fig. 2). All rats in the zolpidem, lorazepam, and TPA023 groups met criterion within a range of 21 to 67 sessions. For these three groups, there was not a significant effect of Training Group in the number of sessions to meet criterion [$F(2,17) = 2.979$, $p = 0.08$]. The range of sessions to criterion for the lorazepam group was, however, usefully narrower (25–34) than for the zolpidem (30–62) and TPA023 (21–67) groups.

After 160 total sessions of discrimination training, including all the manipulations described above, however, rats trained to discriminate TPA023B from vehicle did not meet the performance criteria, and training ended. Although the total percentages of D- or ND-appropriate responding frequently were at criterion level, performance prior to receipt of the first pellet was not. That is, a "win-stay/lose-shift" contingency, coupled with a high probability of always starting on one or the other lever for an individual rat (i.e., "lever preference"),

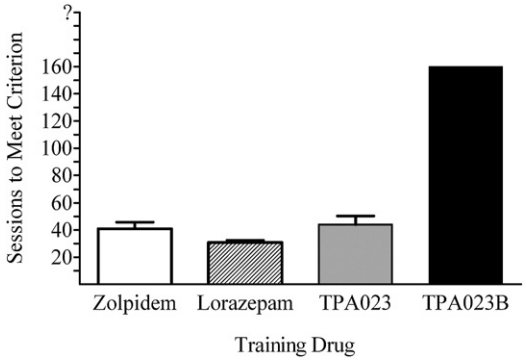

Fig. 2. Mean training sessions to reach the performance criterion for rats in the zolpidem-2.0-mg/kg, lorazepam-1.0-mg/kg, and TPA023-1.0-mg/kg training groups. Assessment began with the first session in which D and ND sessions began alternating after FR 10 had been reached, and ended with the last session that met criterion prior to the back-to-back test sessions with the training drug dose and its vehicle, that defined demonstration of stimulus control by the training drug. Brackets indicate the SEM. The rats in the TPA023B group (3.2–18 mg/kg doses) did not meet the training performance criterion after 160 sessions, and training ended.

appeared to be controlling lever choice. For example, if the response requirement was 10, and the "preferred" lever was the left one, then once > 10 consecutive responses occurred on the left lever without pellet delivery, the rat would shift to the right-hand lever. Thus, the rat would meet the dual performance criterion in half the training sessions and virtually never meet them in the other half. In our experience with other training conditions (including some rats in the other groups in the present study), raising the FR value all at once by 5 usually eliminated win-stay/lose-shift responding (after which the FR could be lowered to 10); but did not in the TPA023B group. The other manipulations described in Methods did not eliminate this pattern either. Another way in which the TPA023B group differed from the others is that the number of training sessions needed before the response requirement initially could be raised to 10 (i.e., once D and ND sessions were in alternation) was greater than for the other groups (i.e., 21 to 62 sessions for the TPA023B group and 10 to 22 for the others).

2.2. Generalization testing with the training drug

Results of generalization testing are shown in Fig. 3. All zolpidem-2.0-mg/kg-trained rats showed full zolpidem generalization at 1.8 and 3.2mg/kg, and two also showed full generalization at 1.0mg/kg. The ED_{50} value was 1.0mg/kg. Response rates dose-dependently decreased to 40% of the rate after vehicle injection at the highest zolpidem dose (ED_{50} was 2.4mg/kg). All lorazepam-1.0-mg/kg-trained rats showed full lorazepam generalization at 0.56mg/kg and higher, and the ED_{50} was 0.42mg/kg. Response rates were not strongly affected by lorazepam in this group. All TPA023-1.0-mg/kg-trained rats showed full generalization at doses of 1.0, 1.8 and 3.2mg/kg; two rats also showed full generalization at 0.32mg/kg; and three showed partial generalization at 0.1mg/kg TPA023. The ED_{50} was 0.42mg/kg. Response rates were not affected by any dose of TPA023.

2.3. Generalization testing with TPA023B

The zolpidem and lorazepam groups did not show full generalization to TPA023B (Fig. 4). In the zolpidem-2.0-mg/kg-trained group, three rats emitted 100% D-lever responses at one of three different doses (0.32, 1.0, and 32mg/kg TPA023B). All other rats showed little or no zolpidem-appropriate responding. In the lorazepam-1.0-mg/kg-trained group, two rats made100% D-lever responses at either 3.2 or 32mg/kg. All other rats showed little or no lorazepam-appropriate responding. In the TPA023-1.0-mg/kg-trained group, four to six rats showed full generalization to TPA023B at 0.32 to 32mg/kg. The ED_{50} value was 0.206mg/kg. Response rates were not strongly affected by TPA023B in any of the three training

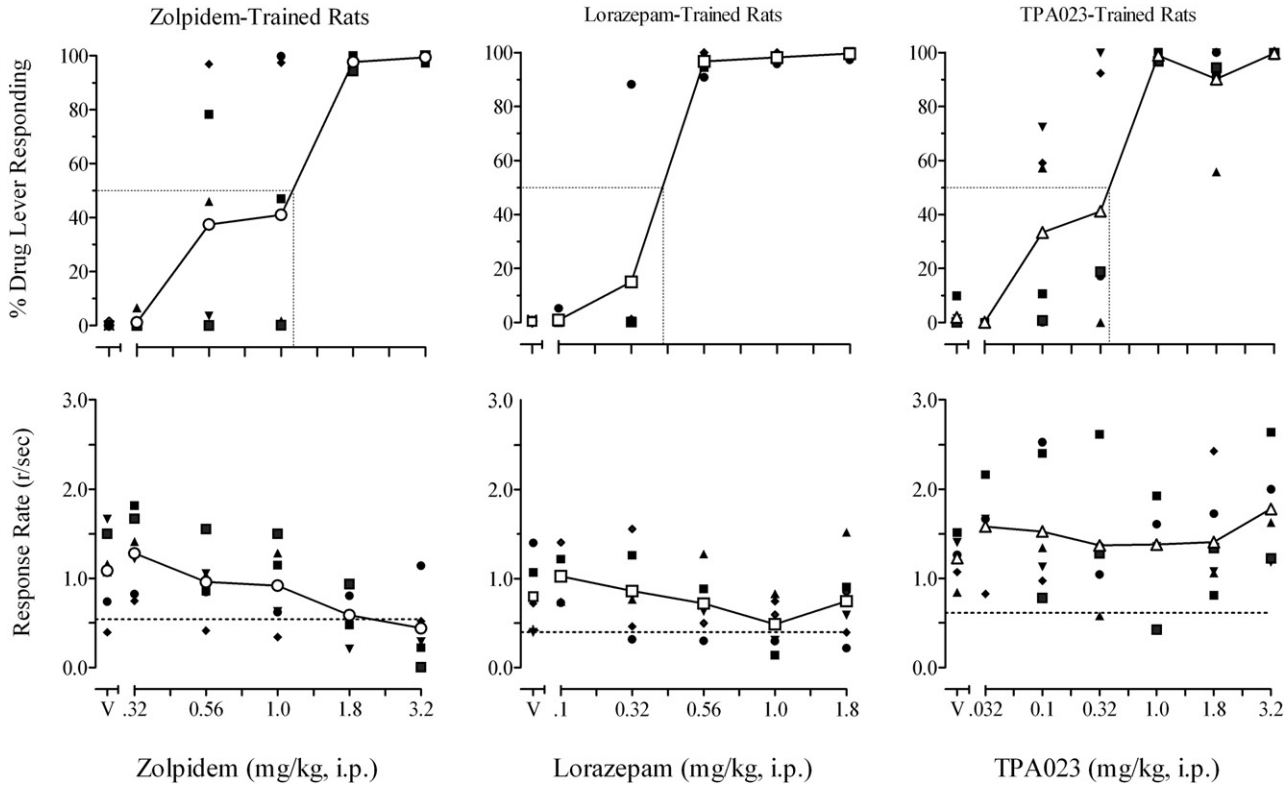

Fig. 3. Upper panels show the mean percentages of responding on the training drug-appropriate lever in test sessions after vehicle (V) and doses of zolpidem, lorazepam, or TPA023 for each training group, respectively. Connected points indicate the group mean, and unconnected points present data for individual rats; different symbols represent different rats. Dotted line indicates ED_{50} for each training dose. Lower panels show the overall mean rate of responding (i.e., on both levers combined) in the same test sessions. Dotted line indicates 50% reduction in rate of responding from the V mean.

groups. The highest TPA023B dose tested was determined by solubility limits of TPA023B in relation to the maximal volume of injection preferred (i.e., 2ml/kg i.p.).

2.4. Performance in training sessions after TPA023B tests

After tests with any dose of TPA023B, rats took strikingly longer than usual to meet the performance criterion in training sessions. Fig. 5 shows the mean sessions to criterion for each training group following tests with other doses of its training drug (panel A) compared to this same measure following tests with doses of TPA023B (panel B), which support this observation and also suggest a TPA023B dose-dependence for the effect. The ANOVA on novel TPA023B dose revealed a significant main effect of Training Drug [$F(2,10) = 5.786$, $p = 0.021$], and a significant main effect of TPA023B Dose tested [$F(4,40) = 9.691$, $p < 0.001$], but no Training Drug-by-Dose-Tested interaction [$F(8,40) = 1.055$, $p = 0.413$]. This is in contrast to the repeated measures ANOVA on novel doses of the training drug, which revealed no main effect of dose in any of the training groups (all F's < 2.576, p's > 0.069). These data suggested that residual TPA023B could have been present 24h after its administration.

The data for individual rats were inspected to determine the nature of the "day-after-TPA023B" performance. When a zolpidem or lorazepam (i.e., D) training session occurred 24h after a TPA023B test session, there was an unusual prevalence of responding on the ND lever before a pellet was obtained for responding on the D lever. For example, in the zolpidem group, with 12 D sessions available for analysis, 4 of the 5 rats generally made more than 10 responses (median = 35) on the ND lever before making 10 consecutive responses on the D lever (mean ND lever responding before the first pellet earned was 51%; median = 61.5%). On at least one D training day, each of 4 zolpidem-trained rats made 82–184 responses on the ND lever; on one occasion each, 2 of them failed to make enough D lever responses to earn even one food pellet in that session. In the lorazepam group, with 15 D sessions available for analysis, 4 of the 5 rats always made more than 10 responses on the ND lever (median = 33) before obtaining the first pellet when lorazepam was given the day after TPA023B (the mean percentage on the ND lever was 59%; median = 68%). On at least one occasion, 4 lorazepam-trained rats made 96–424 responses on the ND lever; the rat that made 424 never made enough responses on the D lever to obtain even one food pellet that session. There were insufficient occasions (i.e., 2 in the zolpidem group and 3 in the lorazepam group) on which an ND training day fell 24h after a TPA023B test to do an analysis of performance in those sessions.

When a TPA023 (D) training session occurred 24h after a TPA023B test, an unusual amount of responding on the ND lever did not occur. With 17 D sessions available for analysis, 5 of the 6 rats usually made less than 10 responses on the ND lever prior to completing 10 consecutive responses on the D lever (median was 3). The group mean percentage on the ND lever for the TPA023 training day after tests with TPA023B was 35% before the first pellet delivery (median was 23%). By comparison, all rats made 0% ND lever responses when their training drug was given after vehicle. When an ND training day fell the day after TPA023B, however, a large number of responses occurred on the D lever before the first pellet was received on the ND lever. With 11 ND sessions available for analysis, all 5 rats for which data could be assessed responded predominantly on the D lever on at least one occasion prior to finally completing the 10-response requirement on the ND lever (mean = 54%; median = 72%).

Thus all three groups' performances suggested that residual drug was present on the day after TPA023B. The patterns of responding suggested that it interfered with the discriminative stimulus effects of zolpidem and lorazepam, but not TPA023. Rather, a TPA023-like discriminative effect was suggested by the performance of the TPA023 group.

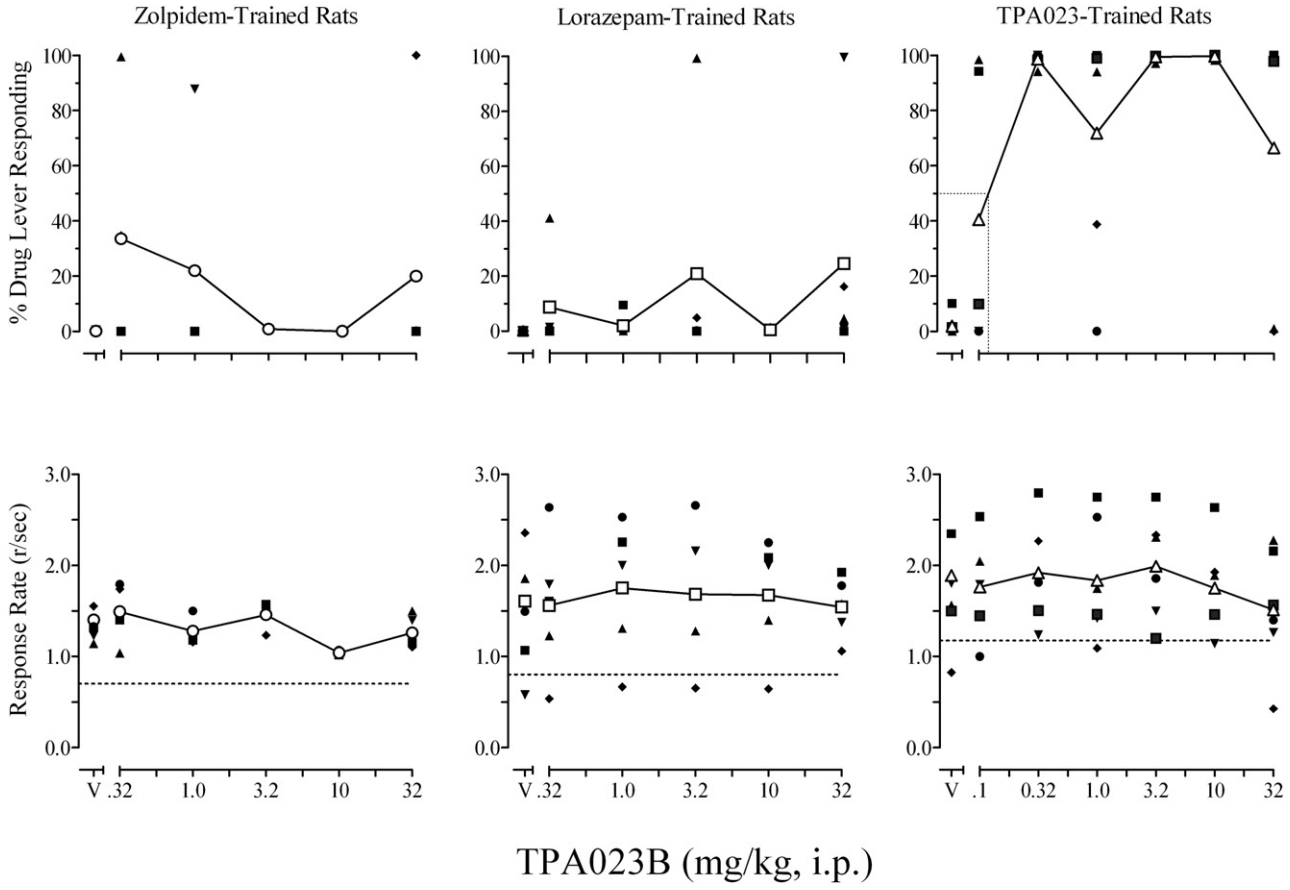

Fig. 4. Results of generalization testing with vehicle (V) and TPA023B in groups of rats trained to discriminate zolpidem, lorazepam, or TPA023. Other details as in Fig. 3.

2.5. Assessment of ataxia

Because subjective assessment had been that rats injected with 18mg/kg TPA023B in that training group had initially felt limp when being handled after the pretreatment, an objective test of muscle relaxation was carried out using the highest dose that had been used in testing TPA023B in lorazepam, zolpidem, and TPA023 groups (i.e., 32mg/kg). The mean (± SEM) numbers of paw slips after vehicle and after TPA023B injections are shown in Fig. 6. TPA023B 32mg/kg significantly increased the number of paw slips compared to vehicle ($t = -2.996$, $p = 0.020$, one-tailed).

3. Discussion

In the present study, TPA023B, a compound with a unique profile of selective, partial-agonist efficacy at BZ/$GABA_A$ receptor subtypes had interoceptive stimulus effects unlike those of prototypical anxiolytic and hypnotic ligands for the BZ recognition site, zolpidem and lorazepam. This suggests that its subjective effects are unlike those prototypical ligands as well. Furthermore the compound is unique in that it did not support being trained as a discriminative stimulus by use of methods that successfully trained zolpidem, lorazepam, and the closely related selective partial agonist TPA023, which was used as a

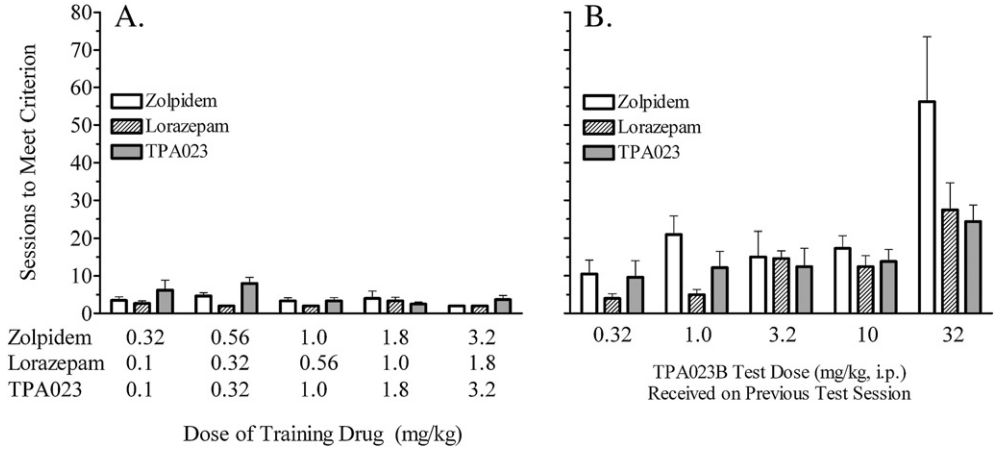

Fig. 5. Mean training sessions to reach the training performance criterion for rats in the zolpidem-2.0-mg/kg, lorazepam-1.0-mg/kg, and TPA023-1.0-mg/kg training groups after novel dose tests with their own training drug (panel A), and after test sessions in which doses of 0.32, 1.0, 3.2, 10, and 32 mg/kg TPA023B had been administered (panel B). Brackets indicate SEM. Note: The lowest novel test dose of TPA023 (0.032 mg/kg) is not shown in that training group in order to maintain consistency between the different training groups.

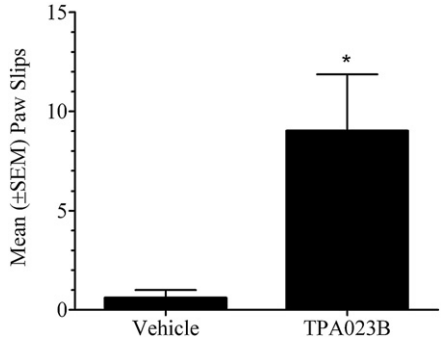

Fig. 6. Mean number of times a rat's paw slipped between the bars of the operant testing chamber 120 min following vehicle and 32 mg/kg TPA023B for the rats in the TPA023B training group. Brackets indicate SEM. *$p<0.05$ by paired *t*-test.

training drug for the first time. That TPA023B does have measurable interoceptive stimulus effects was shown by robust full generalization by rats trained to discriminate TPA023.

3.1. Generalization results

Given the great similarity in the selective, partial-agonist profiles for TPA023 and TPA023B at $\alpha_{1,2,3}$-subunit-containing BZ/$GABA_A$ subtypes, full generalization from TPA023 to TPA023B was predicted. The potency of TPA023B in producing discriminative stimulus effects in TPA023-trained rats was about twice that for generalization to TPA023 itself, which could have been a function of the more than two-fold greater efficacy of TPA023B at the $\alpha_{2,3}$ subtypes. Since the dose of the training drug itself plays a role in the relative potency of test drugs, however, potency as a discriminative stimulus cannot be interpreted solely by the drug's efficacy in GABA modulation (e.g., Ator and Griffiths, 1989).

Lack of generalization by zolpidem-trained rats to TPA023B was predicted if one assumes that zolpidem's preferential binding and full agonist activity at α_1-$GABA_A$ subtypes is prepotent in the interoceptive stimulus of a zolpidem discrimination. Zolpidem selectivity for α_1 $GABA_A$ subtypes is not exclusive but is approximately 10-fold greater than for α_2 subtypes and 14-fold greater than for α_3 (Dämgen and Lüddens, 1999). Binding at $\alpha_{2/3}$ subtypes may play a role in the zolpidem discriminative stimulus, but this activity does not appear to be prominent given the lack of generalization to TPA023B by zolpidem-trained animals. It's also possible that prominent α_5-efficacy for TPA023B, in the context of the rest of its non-zolpidem-like profile, contributed to lack of overlap in discriminative effects, since zolpidem does not have efficacy at that subtype (Dämgen and Lüddens, 1999).

Lack of generalization by lorazepam-trained rats to TPA023B was predicted based on our previous study of the generalization profile for lorazepam-trained animals. Not only had the related compound TPA023 not occasioned lorazepam-appropriate responding in baboons (Ator, 2005), nonselective partial agonists (e.g., U-78875, bretazenil, and imidazenil) have not produced full generalization in lorazepam-trained baboons or rats either. These latter compounds have produced peak group mean percentages of lorazepam-appropriate responding between 40 and 60%, however; but TPA023 and TPA023B did not (Ator and Griffiths, 1999; Ator and Kautz, 2000; Ator, unpublished).

Since TPA023B and TPA023 differ in their GABA-modulating efficacies at $\alpha_{2/3/5}$ subtypes, but are alike in lack of agonist efficacy at α_1-containing subtypes, this latter property may have determined lack of even partial generalization to the TPA compounds by lorazepam-trained baboons or lorazepam- and zolpidem-trained rats. This interpretation is consistent with zolpidem's preferential binding at the α_1 subtype and with the hypothesis that the lorazepam discriminative stimulus has a prominent α_1-mediated component (Ator and Griffiths, 1999; Ator, 2000, 2005). Data obtained 24h after tests with TPA023B indicated that residual TPA023B was present and that it dose-dependently reduced the probability of D lever responding when the zolpidem and lorazepam training doses were given. This outcome is consistent with the prediction that TPA023B's lack of α_1 efficacy makes it an antagonist at that subtype. Thus the "day-after" data further support the hypothesis of a strong role for that subtype in the zolpidem and lorazepam discriminative stimuli. TPA023B is a partial agonist at the other relevant $GABA_A$/BZ subtypes, however, so the day-after data also are consistent with the prediction that a partial agonist will reduce the effect of a full agonist at the same binding site (Kenakin, 1993). Only an interaction study with a selective α_1 antagonist would be definitive regarding the necessary role of the α_1 subtype in the zolpidem and lorazepam discriminative stimuli.

3.2. Response rates and ataxia

Although response rates were not decreased by lorazepam in the present study, this was due to the phenomenon of tolerance; that is, they were decreased early in training (cf., Ator and Griffiths, 1989). Rates were decreased by the higher doses of zolpidem, consistent with reports of failure of tolerance to develop to zolpidem's effects (Sanger and Zivkovic, 1992). The lack of response-rate-decreasing effects for TPA023 and TPA023B in any group of rats may be due either to their partial agonist profiles across $\alpha_{2,3,5}$ $GABA_A$ subtypes or lack of efficacy at α_1, or both. These findings are consistent with the lack of effects of the TPA compounds on mouse rotarod, rat chain pulling, and squirrel monkey lever pressing (Atack et al., 2006; Russell et al., 2006). Related data from mutant mice that lack the α_1 subtype showed that diazepam did not decrease locomotor activity. This result parallels data in wild-type mice administered L-838,417, which, like TPA023 and TPA023B, binds but does not have efficacy at α_1 subtypes (Rudolph et al., 1999; McKernan et al., 2000). Taken together, these data suggest that BZ-agonist effects on trained rates of operant lever pressing and locomotor activity are mediated via the α_1 subtype.

Although the potent discriminative effects of TPA023B in the TPA023-trained rats made clear that behavioral effects could be obtained with TPA023B in the Long–Evans rat, it was important to have evidence of some other behavioral effect in these rats to indicate that behaviorally active doses had been reached in testing the lorazepam- and zolpidem-trained rats. The flaccid muscle tone that had been noted in handling the rats after higher doses of TPA023B prompted choice of the paw slip test, which is presumably a measure of ataxia (Melnick et al., 2002). The paw slip measure was more sensitive to the effects of a high dose of TPA023B than was the rate of food-reinforced lever pressing. Given receptor occupancy data (see below), the occupancy at the 32-mg/kg dose likely was 100%; thus it has been suggested that activity at other sites (e.g., α_4 and/or α_6, John R. Atack, personal communication) may have mediated the effect in the paw slip test. Mediation of muscle relaxation by $\alpha_{2/3}$ subtypes is, though, consistent with previous findings (Rowlett et al., 2005a,b).

3.3. Training the TPA023 and TPA023B discriminations

That the TPA023 discrimination could be readily trained proved that a selective, low-efficacy partial agonist profile does not eliminate discriminability per se. In fact, the prediction was that TPA023B would be easier to train than TPA023 given TPA023B's 3.5-, 2.4-, and 7.4-fold greater efficacy at, respectively, α_1, α_2, and α_3-subunit-containing $GABA_A$ subtypes, coupled with its 37% efficacy at α_5 subtypes compared to 5% for TPA023. Yet TPA023 was trained relatively easily at 1.0mg/kg, while TPA023B was not even at a dose that was 56 times the lowest TPA023B dose to produce full generalization in TPA023-trained rats.

Failure to train a TPA023B discrimination in any rat in 160 sessions could have involved behavioral and/or pharmacological variables. Use of the same training procedures, all carried out by a single experimenter,

was successful in training the zolpidem, lorazepam, and TPA023 discriminations in 21 to 67 sessions, regardless of training drug. In particular, our method of shaping lever pressing in the two-lever chamber only when the reinforced lever was paired with the appropriate pretreatment condition (i.e., ND or D) has been shown to be optimal for speed of acquisition, as has the use of an FR schedule of reinforcement (Overton, 1982; Overton and Hayes, 1984). Furthermore, manipulation of relevant training parameters (dose, pretreatment interval, response requirement) has been successful in training drug discriminations, including difficult ones, in rats (e.g., buspirone, Ator, 1991; cf., Ator and Griffiths 1989). Although increasing response requirement often aids in bringing performance to criterion level, increasing training dose always has been successful in this regard (1982, 1984; Sanger and Zivkovic, 1986; Ator, 1991, unpublished). Thus it was unexpected that doing so with TPA023B was not.

Receptor occupancy must also be considered relative to ease of training. Lorazepam 1.0mg/kg and TPA023 1.0mg/kg BZ-site occupancies were, respectively, 40 and 76% 30min after p.o. administration in rats (Atack et al., 2006, 2007). In the present study, both drugs were trained equally easily at those same doses, albeit with different pretreatment times. TPA023B produced 88% receptor occupancy at 3.0mg/kg in rats 30min after p.o. administration, and that dose produced positive effects in the elevated plus maze (compound 11, Russell et al., 2006). A dose of 3.2mg/kg shared discriminative effects with TPA023 in the present study, yet neither 3.2mg/kg TPA023B nor higher doses could be trained to serve as a discriminative stimulus despite undoubtedly full occupancy of the BZ site at these higher doses. This, coupled with the fact that TPA023B shared discriminative effects with TPA023 suggests that the failure to train TPA023B may be due to some characteristic of the compound other than receptor occupancy.

Pharmacokinetic variables may have contributed to difficulty training the TPA023B stimulus as suggested by the unusual, dose-related, delay shown by the zolpidem, lorazepam, and TPA023 training groups in demonstrating criterion training performance after TPA023B had been administered (see Fig. 5). Residual TPA023B (or a metabolite) may have been present and interfered with stimulus control by the training drug. Only toward the end of the study did we learn that the elimination half-life of TPA023B in rat is 11h, which supported conclusions based on the behavioral data (Russell et al., 2006). Thus residual TPA023B present on ND training days could have interfered with the acquisition of the D vs. ND discrimination for the TPA023B training group. That is, there would not have been a true ND condition. Yet, presumably, a low- vs. high-dose TPA023B discrimination could have been trained, albeit inadvertently, given the literature on such discriminations and our experience (e.g., Sannerud and Ator, 1995). The interpolation of a day off after every TPA023B dose should have helped with the problem of residual drug, but did not. In fact, the "routine" occurrence of days off after drug (e.g., weekends, holidays) did not appear to have any facilitative effect on acquisition. It is possible however, that the systematic manipulation of giving a single day off after administration of TPA023B was not enough to overcome the prolonged effects of the drug. In fact, inspection of the number of days to regain criterion after a TPA023B dose suggests that > 4days may be necessary for drug clearance. This hypothesis could simply, though laboriously, be tested by increasing the number of days between training sessions.

An alternative explanation for failure to train the TPA023B discrimination emerges from the literature on the function of receptor subtypes in mediating particular BZ actions. TPA023B has 37% in vitro efficacy at α_5 subtypes. Agonist activity at α_5 subtypes appears to play an important, although not exclusive, role in BZ amnestic effects, while antagonist activity enhances learning (Collinson et al., 2002; Dawson et al., 2005b; cf., Mintzer and Griffiths, 1999). Given the undoubtedly high receptor occupancy of the training doses of TPA023B, its α_5-agonist activity may have interfered with learning in D training sessions. This effectively could have stymied acquiring the discrimination itself, because the experience of differential reinforcement for responding on each of the response options juxtaposed across conditions in which different stimuli reliably are or are not present is necessary to achieve discriminative stimulus control. If the interoceptive stimulus produces strong amnestic effects, then learning differential contingencies on right- and left-lever responding in D sessions may have been impaired. That this phenomenon does not occur in full agonist ligands for the BZ site, particularly those associated with amnestic effects (e.g., triazolam, Ator and Griffiths, 1989), could be due to their potent hypnotic effects, presumably α_1 mediated, that tend to "cap" doses used to train a BZ discrimination. Thus, it may be the case that 3.2mg/kg (the lowest dose of TPA023B used for training) may have produced amnestic effects on learning from the outset. Since TPA023B was fully efficacious in TPA023-trained rats at the low dose of 0.32mg/kg, a lower dose of TPA023B may be effective in training the discrimination. Clearly further research with TPA023B in tests of learning and memory would shed light on the validity of the idea that an α_5-subunit effect thwarted training the TPA023B discrimination.

3.4. Conclusion

TPA023 and TPA023B both showed effects predictive of anxiolytic efficacy in preclinical testing (Atack et al., 2006; Russell et al., 2006). The present results are particularly of interest in relation to potential development of a therapeutic drug that will be as clinically useful as BZs in outpatient treatment of anxiety disorders but devoid of sedative side-effects and effects that subserve BZ-like abuse liability. The present results, in the context of the failure of TPA023 to maintain self-administration (Ator, 2005), suggest that elimination of α_1 efficacy and strong reduction of $\alpha_{2/3}$ efficacy reduces the subjective effects that may subserve BZ-like reinforcing and subjective effects. Further research is needed to determine whether alternative profiles may achieve the same outcome.

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References

Atack JR. Anxioselective compounds acting at the GABA(A) receptor benzodiazepine binding site. Curr Drug Targets CNS Neurol Disord 2003;2:213–32.

Atack JR, Wafford KA, Tye SJ, Cook SM, Sohal B, Pike A, et al. TPA023 [7-(1,1-dimethylethyl)-6-(2-ethyl-2*H*-1,2,4-triazol-3-ylmethoxy)-3-(2-fluor ophenyl)-1,2,4-triazolo[4,3-*b*] pyridazine], an agonist selective for alpha2- and alpha3-containing GABAA receptors, is a nonsedating anxiolytic in rodents and primates. J Pharmacol Exp Ther 2006;316:410–22.

Atack JR, Scott-Stevens P, Beech JS, Fryer TD, Hughes JL, Cleij MC, et al. Comparison of lorazepam [7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one] occupancy of rat brain γ-aminobutyric $acid_A$ receptors measured using in vivo [^{3}H]flumazenil (8-fluoro 5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic acid ethyl ester) binding and [^{11}C]flumazenil micro-positron emission tomography. J Pharmacol Exp Ther 2007;320:1030–7.

Ator NA. Discriminative stimulus effects of the novel anxiolytic buspirone. Behav Pharmacol 1991;2:3–14.

Ator NA. Zaleplon and triazolam: drug discrimination, plasma levels, and self-administration in baboons. Drug Alcohol Depend 2000;61:55–68.

Ator NA. Contributions of GABAA receptor subtype selectivity to abuse liability and dependence potential of pharmacological treatments for anxiety and sleep disorders. CNS Spectr 2005;10:31–9.

Ator NA, Griffiths RR. Differential generalization to pentobarbital in rats trained to discriminate lorazepam, chlordiazepoxide, diazepam, or triazolam. Psychopharmacology 1989;98:20–30.

Ator NA, Griffiths RR. Drug discrimination analysis of partial agonists at the benzodiazepine site. I. Differential effects of U-78875 across training conditions in baboons and rats. J Pharmacol Exp Ther 1999;289:1434–46.
Ator NA, Griffiths RR. Principles of drug abuse liability assessment in laboratory animals. Drug Alcohol Depend 2003;70:S55–72.
Ator NA, Kautz MA. Differentiating benzodiazepine- and barbiturate-like discriminative stimulus effects of lorazepam, diazepam, pentobarbital, imidazenil and zaleplon in two- versus three-lever procedures. Behav Pharmacol 2000;11:1–14.
Ator NA, Weerts EM, Kaminski BJ, Kautz MA, Griffiths RR. Zaleplon and triazolam physical dependence assessed across increasing doses under a once-daily dosing regimen in baboons. Drug Alcohol Depend 2000;61:69–84.
Bond A. The risks of taking benzodiazepines. In: Hallstrom C, editor. Benzodiazepine dependence. New York: Oxford University Press; 1993. p. 34–45.
Collinson N, Kuenzi F, Jarolimek W, Maubach KA, Cothliff R, Sur C, et al. Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the 5 subunit of the GABAA receptor. J Neurosci 2002;22:5572–80.
Dämgen K, Lüddens H. Zaleplon displays selectivity to recombinant $GABA_A$ receptors different from zolpidem, zopiclone and benzodiazepines. Neurosci Res Commun. 1999;25:139–48.
Dawson GR, Collinson N, Atack JR. Development of subtype selective GABAA modulators. CNS Spectr 2005a;10:21–7.
Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, MacLeod AM, et al. An inverse agonist selective for a5 subunit-containing $GABA_A$ receptors enhances cognition. J Pharmacol Exp Ther 2005b;316:1335–45.
Doble A, Martin IL. Multiple benzodiazepine receptors: no reason for anxiety. Trends Pharmacol Sci 1992;13:76–81.
Goudie AJ, Leathley MJ. Drug-discrimination assays. In: Sahgal A, editor. Behavioral neuroscience Volume II: a practical approach. New York: Oxford University Press; 1993. p. 145–67.
Griffiths RR, Sannerud CA, Ator NA, Brady JV. Zolpidem behavioral pharmacology in baboons: self-injection, discrimination, tolerance and withdrawal. J Pharmacol Exp Ther 1992;260:1199–208.
Hadingham KL, Wingrove P, Le Bourdelles B, Palmer KJ, Ragan CI, Whiting PJ. Cloning of cDNA sequences encoding human α2 and α 3 γ-aminobutyric $acid_A$ receptor subunits and characterization of the benzodiazepine pharmacology of recombinant α 1-, α 2-, α 3- and α 5-containing human g-aminobutyric $acid_A$ receptors. Mol Pharmacol 1993;43:970–5.
Kenakin T. Pharmacologic analysis of drug–receptor interactions. 2nd ed. New York: Raven Press; 1993.
Klepner CA, Lippa AS, Benson DI, Sano MC, Beer B. Resolution of two biochemically and pharmacologically distinct benzodiazepine receptors. Pharmacol Biochem Behav 1979;11:457–62.
Lader M. Historical development of the concept of tranquillizer dependence. In: Hallstrom C, editor. Benzodiazepine dependence. New York: Oxford University Press; 1993. p. 46–57.
Langer SZ, Arbilla S. Imidazopyridines as a tool for the characterization of benzodiazepine receptors: a proposal for a pharmacological classification as omega receptor subtypes. Pharmacol Biochem Behav 1988;29:763–6.
Langer SZ, Arbilla S, Tan S, Lloyd KG, George P, Allen J, et al. Selectivity for omega-receptor subtypes as a strategy for the development of anxiolytic drugs. Pharmacopsychiatry 1990;23(Suppl 3):103–7.
McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, et al. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. Nat Neurosci 2000;3:587–92.
Melnick SM, Rodriguez JS, Bernardi RE, Ettenberg A. A simple procedure for assessing ataxia in rats: effects of phencyclidine. Pharmacol Biochem Behav 2002;72:125–30.
Mintzer MZ, Griffiths RR. Triazolam and zolpidem: effects on human memory and attentional processes. Psychopharmacology 1999;144:8–19.
Möhler H, Fritschy JM, Rudolph U. A new benzodiazepine pharmacology. J Pharmacol Exp Ther 2002;300:2–8.
Overton DA, Hayes MW. Comparison of the degree of discriminability of various drugs using the T-maze drug discrimination paradigm. Psychopharmacology 1982;76:385–95.
Overton DA. Optimal training parameters in the two-bar fixed-ratio drug discrimination task. Pharmacol Biochem Behav 1984;21:19–28.
Pritchett DB, Luddens H, Seeburg PH. Type I and type II GABAA-benzodiazepine receptors produced in transfected cells. Science 1989;245:1389–92.
Rowlett JK, Cook JM, Duke AN, Platt DM. Selective antagonism of $GABA_A$ receptor subtypes: an in vivo approach to exploring the therapeutic and side effects of benzodiazepine-type drugs. CNS Spectr 2005a;10:40–8.
Rowlett JK, Platt DM, Lelas S, Atack JR, Dawson GR. Different $GABA_A$ receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates. Proc Natl Acad Sci USA 2005b;102:915–20.
Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, Fritschy JM, et al. Benzodiazepine actions mediated by specific gamma-aminobutyric acidA receptor subtypes. Nat Neurosci 1999;401:796–800.
Russell MG, Carling RW, Street LJ, Hallett DJ, Goodacre S, Mezzogori E, et al. Discovery of imidazo[1,2-*b*][1,2,4]triazines as GABA(A) alpha2/3 subtype selective agonists for the treatment of anxiety. J Med Chem 2006;49:1235–8.
Sanger DJ, Zivkovic B. The discriminative stimulus properties of zolpidem, a novel imidazopyridine hypnotic. Psychopharmacology 1986;89:317–22.
Sanger DJ, Zivkovic B. Differential development of tolerance to the depressant effects of benzodiazepine and non-benzodiazepine agonists at the omega (BZ) modulatory sites of GABAA receptors. Neuropharmacology 1992;31:693–700.
Sannerud CA, Ator NA. Drug discrimination analysis of midazolam under a three-lever procedure I: Dose-dependent differences in generalization and antagonism. J Pharmacol Exp Ther 1995;272:100–11.
Sidman M. A note on the measurement of conditional discrimination. J Exp Anal Behav 1980;33:285–9.
Sieghart W, Sperk G. Subunit composition, distribution and function of GABA(A) receptor subtypes. Curr Top Med Chem 2002;2:795–816.
Weerts EM, Ator NA, Grech DM, Griffiths RR. Zolpidem physical dependence assessed across increasing doses under a once-daily dosing regimen in baboons. J Pharmacol Exp Ther 1998;285:41–53.
Whiting PJ. GABA-A receptor subtypes in the brain: a paradigm for CNS drug discovery? Drug Discov Today 2003;8:445–50.