Published in final edited form as:
Psychopharmacology (Berl). 2011 May ; 215(2): 311–319. doi:10.1007/s00213-010-2142-y.

Contribution of $GABA_A$ Receptors Containing α3 Subunits to the Therapeutic-Related and Side Effects of Benzodiazepine-Type Drugs in Monkeys

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Abstract

Rationale—Experimental evidence suggests that the differential behavioral effects of benzodiazepines depend on their relative actions at γ-aminobutyric acid type A ($GABA_A$) receptors that contain either an α1, α2, α3 or α5 subunit.

Objectives—The present study was aimed at understanding the role of α3 subunit-containing $GABA_A$ ($\alpha3GABA_A$) receptors by examining the behavioral pharmacology of TP003 (4,2'-difluoro-5'-[8-fluoro-7-(1-hydroxy-1-methylethyl)imidazo[1,2-a]pyridine-3-yl]biphenyl-2-carbonitrile), which shows functional selectivity for $\alpha3GABA_A$ receptors.

Methods—First, a conflict procedure was used to assess the anxiolytic-like effects of TP003 and a representative clinically available benzodiazepine. TP003 was also administered before daily periods of sucrose pellet availability to evaluate potential hyperphagic effects. In separate experiments, observable behavioral effects were used to assess the motor and sedative effects of TP003.

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None of the authors have competing personal financial interests that may be influenced by the publication of this report.

Results—Administration of TP003 produced robust anti-conflict effects without the rate-decreasing effects that were observed with the representative benzodiazepine. Unlike reported effects of benzodiazepines, TP003 did not enhance palatable food consumption. However, increases in observable sleep-associated posture were induced by TP003, as were decreases in some species-typical behaviors (vocalization, locomotion, and environment-directed behaviors). When evaluated for its ability to induce a procumbent posture, TP003 failed to produce an effect.

Conclusions—Based on conflict and observation tests in monkeys, our results suggest that TP003 may have anxiolytic properties but lacks ataxic, hyperphagic, and pronounced sedative effects characteristic of classical benzodiazepines. TP003 did induce myorelaxant-like effects and had relatively mild sedative effects. Collectively, these results suggest that α3$GABA_A$ receptors play an important role in the anxiolytic-like and motor effects of benzodiazepine-type drugs.

Keywords

$GABA_A$ receptors; α3 subunit; benzodiazepine; anxiety; sedation; ataxia; myorelaxation; hyperphagia

INTRODUCTION

The γ-aminobutyric acid type A ($GABA_A$) receptors are the primary sites of action for benzodiazepines and related drugs used to treat anxiety and sleep disorders. The therapeutic use of benzodiazepine-type drugs for the treatment of these disorders is constrained, however, by the occurrence of other characteristic effects that are mediated through $GABA_A$ receptors. In addition to their therapeutic effects, benzodiazepines produce unwanted side-effects including daytime drowsiness, impairment of motor coordination, and increases in food consumption.

Benzodiazepine-type drugs act by binding allosterically to a distinct site on $GABA_A$ receptors which produces changes that enhance the ability of GABA to increase chloride conductance. Research during the past two decades has revealed the existence of multiple subtypes of the $GABA_A$ receptor (e.g., Pritchett et al. 1989; Rudolph et al. 2001). Subsequent reports have postulated that the diverse behavioral effects of benzodiazepine-type drugs may reflect actions at different subtypes of $GABA_A$ receptors (Rudolph et al. 1999; McKernan et al. 2000; Löw et al. 2000; Rowlett et al. 2005).

$GABA_A$ receptors in the central nervous system are pentamers constituted from structurally distinct proteins, with each protein family consisting of different subunits (for review, see Rudolph et al. 2001). The majority of $GABA_A$ receptors are composed of α, β, and γ subunits and benzodiazepines bind to a site on the native $GABA_A$ receptor that is located at the interface of the γ2 subunit and one of the α1, α2, α3, or α5 subunits. Benzodiazepines do not bind to the corresponding α4- and α6-subunit containing receptors. Approximately 75% of the $GABA_A$ receptors in the brain contain α1, α2, and α3 subunits (McKernan and Whiting, 1996), and $GABA_A$ receptors containing α1 subunits (α1$GABA_A$ receptors) recently have been implicated in the sedative effects of benzodiazepines, whereas $GABA_A$ receptors containing α2 and α3 subunits (α2$GABA_A$ and α3$GABA_A$ receptors) are associated with the anxiolytic and myorelaxant effects of benzodiazepines (McKernan et al. 2000; Löw et al. 2000; Rowlett et al. 2005, Morris et al. 2006). $GABA_A$ receptors containing α5 subunits (α5$GABA_A$ receptors), in contrast, are a relatively minor population in the brain as a whole but are preferentially expressed within the hippocampus and play a role in certain memory processes, but are not responsible for the anxiolytic or motor effects associated with benzodiazepines (Collinson et al. 2002; Crestani et al. 2002; Atack et al. 2006; but see Savic et al. 2008).

Pharmacological efforts to attribute the contribution of $GABA_A$ receptor subtypes to the multiple effects of benzodiazepines have been enhanced in recent years by the increasing availability of compounds with selectivity for the individual receptor subtypes. In this regard, Dias et al. (2005) described an imidazopyridine compound, TP003, which exhibits "functional selectivity" rather than binding selectivity for $GABA_A$ receptor subtypes containing α3 subunits. That is, *in vitro* TP003 has comparatively high agonist efficacy at $\alpha3GABA_A$ receptors, but essentially no efficacy at $\alpha1GABA_A$, $\alpha2GABA_A$, and $\alpha5GABA_A$ receptors (Dias et al. 2005). Because TP003 exhibits appreciable efficacy only at $\alpha3GABA_A$ receptors, the extent to which this compound induces an effect characteristic of conventional benzodiazepines can be interpreted as evidence for a specific role of $\alpha3GABA_A$ receptors in that particular effect.

Using this approach in the present study, we evaluated the ability of TP003 to engender characteristic anxiolytic-like, hyperphagic, motor and sedative effects in relevant non-human primate models of the therapeutic and side effects of benzodiazepines (Platt et al. 2002; Licata et al. 2005; Duke et al. 2006; Rowlett et al. 2006). The anxiolytic-like effects of TP003 were first assessed in a conflict procedure in which behavior was maintained under a fixed-ratio schedule of food delivery in the absence (non-suppressed responding) and presence (suppressed responding) of response-contingent electric shock. In addition, hyperphagic effects were assessed by administering TP003 before 10-min periods of sucrose availability. Finally, previously validated observational techniques were used to assess the motor and sedative effects of TP003. The behavioral effects of TP003 are discussed in relation to previous studies assessing both conventional benzodiazepines and subtype-selective $GABA_A$ receptor agonists.

MATERIALS AND METHODS

Animals

Subjects were adult rhesus monkeys (*Macaca mulatta*) for the conflict studies, and adult squirrel monkeys (*Saimiri sciureus*) for the sucrose pellet consumption and observation studies. Separate groups of monkeys were used fo each procedure. Monkeys in the conflict studies were maintained at 90–95% of their free-feeding weights, the other monkeys were not food restricted. Monkeys were housed individually and maintained on a 12-hr lights-on/12-hr lights-off cycle (lights on at 0600 hr), with water available continuously. All testing occurred during the lights-on phase of the cycle. Rhesus monkeys were prepared with a chronic indwelling venous catheter according to the procedures described by Platt et al. (2005). Animals in this study were maintained in accordance with the "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council 2003) and the principles of laboratory animal care were adhered to.

Conflict Procedure

Four rhesus monkeys (2 males, 2 females) were trained as described in detail by Rowlett et al. (2006). A daily session consisted of 4 cycles, each preceded by a 10-min time out period in which all lights in the chamber were off and responding had no programmed consequences. Each cycle consisted of two components. The first component was signaled by red stimulus lights and consisted of a fixed ratio (FR) 18 schedule of food pellet delivery (Bioserve, Frenchtown, NJ) followed by a 10 s time out. The second component, signaled by green stimulus lights, consisted of the FR 18 schedule of food delivery combined with a FR 20 schedule of foot shock delivery (1.5 – 3.0 mA, adjusted for each monkey based on individual performance, 0.25 s duration). Both components were 5 min in duration, or ended after the monkey obtained 5 food pellets or received 3 foot shocks, whichever occurred first.

Test sessions were conducted once or twice per week. Here, i.v. injections of vehicle or drug were administered in the 5th minute of each time out. In successive cycles, increasing doses of the test drug were administered using a cumulative dosing procedure. The dependent measure was the average rates of responding (responses/s), calculated by dividing responses by time during components 1 and 2, excluding responding during time outs or reinforcer delivery.

Sucrose pellet consumption

Sucrose pellet consumption was evaluated as described in Duke et al. (2006). Briefly, male squirrel monkeys (N=5) were placed in the observation area once a week with access to a dish containing 100 sucrose pellets (P.J. Noyes, Lancaster, NH) for 10 min. Drugs or vehicle were administered intramuscularly (i.m.) 15 min prior to each test session. Sucrose pellet consumption was measured by subtracting the number of pellets remaining in the food dish or elsewhere in the observation chamber from 100.

Observation

Five male squirrel monkeys were habituated to an observation arena and injection procedures (described in Platt et al. 2002) for approximately one month. Following habituation, 30-min observational sessions were conducted following a 15 min pretreatment of drug or vehicle. During the sixth, eighteenth and thirtieth min of each 30-min session, the monkeys were removed briefly from the observation arena by a trained handler and evaluated for ataxia, defined as the inability to balance on a stainless steel transport pole held in the horizontal plane. During each ataxia assessment, a score of 0 indicated that the monkey was able to balance normally on the transport pole, a score of 1 indicated inability to balance, and a score of 2 indicated that the monkey could neither balance on nor support its weight on the pole. In addition, a measurement of muscle resistance was taken in order to determine myorelaxant effects (cf. Licata et al. 2009). After rating the ability of the monkey to balance on the pole, the experimenter then grasped one leg and gently extended it to assess the degree of resistance to flexion. A score of 0 indicated that the monkey retracted its leg normally, a score of −1 indicated delayed and/or reduced resistance to leg flexion, and a score of −2 indicated no flexion of the leg.

Scoring of videotapes was conducted by observers trained to use the behavioral scoring system described by Platt et al. (2002). Eight behaviors (Platt et al. 2002) were scored by recording their presence or absence in 15-s intervals during three 5-min observation periods across the session (0–5 min, 12–17 min, 24–29 min). Frequency scores were calculated from these data as the proportion of 15-s intervals in which a particular behavior occurred, and the maximum possible score was 20.

Data analysis and drug preparation

Effects of doses of compounds were evaluated by conducting *a priori* Bonferroni t-tests (parametric data) or Dunn's *Q* statistic (non-parametric data), comparing individual doses to vehicle injection. Potency values (dose engendering 50% maximum effect, ED_{50}) were calculated in individual monkeys by log-linear regression analysis. For all comparisons, the alpha level was set at $p \leq 0.05$.

TP003 was provided by Merck, Sharp, & Dohme Research Laboratories (Harlow, UK), and was prepared in a vehicle of 10% benzyl alcohol, 50% propylene glycol, and 40% sterile water. All other drugs were purchased from Tocris-Cookson (Ellisville, MO, USA), and were dissolved in 50% propylene glycol, 50% sterile water.

RESULTS

Conflict

Figure 1a shows the effects of TP003 on the fixed-ratio schedule of food pellet delivery (non-suppressed responding) and the concurrent schedule of food delivery and electric shock presentation (suppressed responding). Following vehicle administration, rates of responding during both components were similar to those observed during training sessions (i.e. between 2.0–3.0 responses/s during the non-suppressed component, and less that 0.1 responses/s during the suppressed component). TP003 increased the mean rates of suppressed responding compared to vehicle at doses of 1.0 to 5.6 mg/kg (Bonferroni *t* tests, $p<0.05$) with an ED_{50} value of 0.53 mg/kg, i.v.. Across the doses tested, TP003 did not affect response rates during the non-suppressed component.

We also determined the anti-conflict effects of alprazolam as a comparative standard. Administration of alprazolam engendered a characteristic increase in the rates of suppressed responding at low to intermediate doses and attenuated the rates of non-suppressed responding at higher doses (Figure 1b), and the ED_{50} values from each component is included in Table 1. As can be seen in the table, alprazolam was ~45-fold more potent than TP003, and attenuated non-suppressed responding at doses ~10-fold higher than those that increased rates of suppressed responding. For comparison purposes, the previously reported potencies of HZ-166 and zolpidem, two subtype-selective drugs, are also shown in Table 1.

Ataxia and myorelaxation

The ataxic and myorelaxant effects of TP003 as assessed in the quantitative observational procedures are shown in Figure 2. Administration of vehicle did not engender ataxic or myorelaxant effects in any monkey. TP003 also did not increase ataxia scores across the dose range tested. However, dose-dependent decreases in muscle resistance scores were observed after TP003 administration, with an effect significantly different from vehicle administration at 1.0 mg/kg (Dunn's *Q*, $p<0.05$). The median scores from the maximally effective dose of TP003, and for comparison the previously reported scores of alprazolam and zolpidem, are shown in Table 2.

Sucrose pellet consumption

The hyperphagic effects of TP003, as determined in the assay of sucrose-pellet consumption, are shown in Figure 3a. Under baseline conditions, monkeys consumed a mean (± S.E.M.) of 19 ± 4 sucrose pellets during the 10-min access period. Sucrose pellet consumption was not altered following administration of vehicle. Across the doses tested, TP003 also did not significantly affect the consumption of sucrose pellets. The percent increase in sucrose pellet consumption following administration of TP003, and for comparison alprazolam and zolpidem, are shown in Table 3.

Sedative-motor effects

Figure 3 also shows observable behavioral effects of TP003 in squirrel monkeys. Dose-dependent decreases in vocalization (Figure 3b, minimum effective dose = 0.3 mg/kg), locomotion (Figure 3c, minimum effective dose = 1.0 mg/kg) and environment-directed behavior (Figure 3d, minimum effective dose = 0.1 mg/kg) were observed following administration of TP003 (Bonferroni *t* tests, $p<0.05$). The frequency measures of rest and procumbent posture induced by TP003 are also shown in Figure 3. TP003 dose-dependently increased the frequency of rest posture (Figure 3e, minimum effective dose = 1.0 mg/kg; Bonferroni *t* tests, $p<0.05$). In contrast, increases in procumbent posture were not observed across the doses tested (Figure 3f). The mean frequency scores for passive visual and self-directed behaviors (a combination of scores for grooming and scratching) were not altered

by TP003 across the dose range tested (0.1–1.0 mg/kg, data not shown). The behavioral frequency scores that were engendered by the maximally effective dose of TP003, and for comparison the previously reported scores of alprazolam and zolpidem, are also shown in Table 3.

DISCUSSION

Conventional benzodiazepines bind non-selectively at $GABA_A$ receptors containing α1, α2, α3 and α5 subunits; however the role of different $GABA_A$ receptor subtypes in the behavioral effects of these drugs has not been characterized fully. We now demonstrate that TP003, a novel ligand with functional selectivity at α3$GABA_A$ receptors, is effective in behavioral measures of the anxiolytic and myorelaxant effects of benzodiazepines in monkeys. TP003 also engendered rest posture, reduced locomotion, and environment-directed behaviors; a profile of effects that raise the possibility of a role of α3$GABA_A$ receptors in relatively mild sedative effects induced by benzodiazepines. In contrast, TP003 was ineffective in behavioral assays of the ataxic, hyperphagic and more pronounced sedative effects (i.e., procumbent posture) of benzodiazepine-type drugs. Together, these findings provide evidence that sets apart the profile of behavioral effects induced by selective α3$GABA_A$ receptor stimulation vs. the profile of behavioral effects engendered by conventional and other subtype-selective benzodiazepines.

Procedures that assess the effects of drugs on experimentally-induced conflict are used often to assess the potential anxiolytic effects of these drugs as in humans (Geller and Seifter 1962; Spealman 1979; Kleven and Koek 1999; Rowlett et al. 2006). Previous studies from our laboratory have used a conflict procedure developed for rhesus monkeys to assess the anxiolytic effects of conventional benzodiazepines and other positive $GABA_A$ receptor modulators with either selective affinity or selective efficacy for $GABA_A$ receptor subtypes (Rowlett et al. 2005; Rowlett et al. 2006; Fischer et al. 2010). Results from these studies provide evidence for a differential role of $GABA_A$ receptors in the anxiolytic effects of benzodiazepines. As an example, L-838,417, a compound with functional selectivity at α2$GABA_A$, α3 $GABA_A$, and α5 $GABA_A$ receptors, produced an anti-conflict effect similar to conventional non-selective benzodiazepines (Rowlett et al. 2005). A similar result was observed when XHe-II-053 and HZ-166, drugs with high intrinsic efficacy at α2$GABA_A$ and α3$GABA_A$ receptors, were assessed in the conflict procedure (Fischer et al. 2010). Together with data suggesting that drugs selective for α1$GABA_A$ receptors (e.g. zolpidem) are only marginally effective in this procedure (Rowlett et al. 2005, 2006), these experiments support a key role for α2$GABA_A$ and α3$GABA_A$ receptors, but not α1$GABA_A$ receptors, in benzodiazepine-induced anxiolysis. Subsequent demonstrations of anxiolytic-like effects after administration of the partial α2$GABA_A$ and α3$GABA_A$ receptor agonist TPA023 in other rodent and primate models of anxiety also support this hypothesis (Atack et al. 2006).

In the present study, administration of TP003 produced an anti-conflict effect to the same degree as observed with the conventional benzodiazepine alprazolam, (see also Rowlett et al. 2006). These data provide clear evidence that a compound with selective efficacy at α3$GABA_A$ receptors can produce anxiolytic-like effects in primates, and support a role for this receptor subtype in the anxiolytic effects of benzodiazepines. It is noteworthy that TP003 produced an anti-conflict effect without altering rates of non-suppressed responding over the dose range tested. Similar results were observed when L-838,417 was assessed in the conflict procedure (Rowlett et al. 2005). Interestingly, both TP003 and L-838,417 are without appreciable efficacy at α1$GABA_A$ receptors, raising the possibility that α1$GABA_A$ receptors may be involved in the response rate-reducing effects of benzodiazepines.

Using previously described techniques (Licata et al. 2009), the present study characterized the motor-altering effects of TP003 across measures related to ataxia and myorelaxation. Studies with mutant mice implicate $\alpha 1GABA_A$ receptors in the ataxic effects of benzodiazepines, and further suggest that $\alpha 2GABA_A$ and $\alpha 3GABA_A$ receptors are involved in benzodiazepine-induced myorelaxation (Rudolph et al. 1999; McKernan et al. 2000; Crestani et al. 2001). Subsequent pharmacological studies in monkeys with subtype-selective $GABA_A$ receptor agonists agree with these earlier findings. (Platt et al. 2002; Licata et al. 2005; Rowlett et al. 2005; Licata et al. 2009). For example, whereas both SL651498 and L-838,417 induce myorelaxant effects, neither drug was effective in producing ataxic effects in the same animals (Licata et al. 2005; Rowlett et al. 2005). Moreover, the ataxic, but not myorelaxant effects of benzodiazepine-type compounds were blocked by the $\alpha 1GABA_A$-preferring antagonist, βCCT (Licata et al. 2009). The lack of ataxic effects observed with TP003 in the present study is consistent with the idea that $\alpha 3GABA_A$ receptors do not play a crucial role in benzodiazepine-induced ataxia. Moreover, the results presented here are consistent with the idea that stimulation of $\alpha 3GABA_A$ receptors alone may be sufficient to produce benzodiazepine-associated myorelaxant effects.

The hyperphagic effects of benzodiazepines have been well documented (e.g. Randall et al. 1960; Cooper and Estall 1985), with cases of night-time bingeing being of particular concern. Studies in animal models have demonstrated increases in food consumption under controlled laboratory conditions, and suggest that different $GABA_A$ receptor subtypes may mediate benzodiazepine-induced hyperphagia. Further, these studies suggest that the $GABA_A$ receptor subtype mediating this effect may be species dependent. For example, $\alpha 2GABA_A$ and $\alpha 3GABA_A$ receptors are important mediators of benzodiazepine-induced hyperphagia in rodents, while $\alpha 1GABA_A$ and $\alpha 5GABA_A$ receptors do not play a role (Yerbury and Cooper 1989, Cooper and Ridley 2005, Stephens et al. 2005, Morris et al. 2009). In contrast, an important role for $\alpha 1GABA_A$ receptors, but not $\alpha 5GABA_A$ receptors as mediators of benzodiazepine-induced hyperphagia has been documented in primates (Wettstein and Spealman 1986, Kumar et al. 1999, Duke et al. 2006). To our knowledge, little information exists on the role of $\alpha 3GABA_A$ receptors in this effect in primates. In the present study, TP003 failed to increase the consumption of sucrose pellets at doses that engendered other behavioral effects (e.g., myorelaxant-like effects) and under conditions in which both conventional and $\alpha 1GABA_A$-preferring agonists produce approximately 300% increases relative to baseline consumption (Duke et al. 2006). These observations suggest that $\alpha 3GABA_A$ receptors do not play a key role in the increases in palatable food consumption observed following conventional benzodiazepine administration, and provide further evidence that the contribution of $GABA_A$ receptor subtypes in this effect may be species dependent.

Quantitative observational techniques (cf. Platt et al. 2002) were used to assess the effects of TP003 on behaviors typically associated with motor impairment and sedation, as well as vocalizations. Previous findings from our laboratory have demonstrated that conventional benzodiazepines typically have no effects on vocalizations but decrease the frequency of locomotor and environment-directed behaviors (e.g. Platt et al. 2002; Licata et al. 2005). Non-selective benzodiazepines also characteristically induce both rest and procumbent posture (i.e., loose-limbed, sprawled, unable to maintain an upright position), the latter suggestive of a more pronounced, deep sedative effect (Platt et al. 2002; Licata et al. 2005; Rowlett et al. 2005; Duke et al. 2006). In contrast to the characteristic behavioral profile associated with non-selective benzodiazepines, TP003 decreased vocalizations and rest posture with no associated increases in procumbent posture over the dose range tested. Interestingly, the behavioral profile of TP003 also differed from the previously-evaluated functionally selective compounds L-838,417 and SL651498, which did not decrease vocalization, locomotion, or environment-directed behavior, and did not engender rest and

procumbent posture. Of particular interest is the lack of rest posture associated with SL651498, which is a full agonist at both α2GABA$_A$ and α3GABA$_A$ receptor subtypes (Licata et al. 2005). Although clearly speculative, one intriguing possibility is that action at α2GABA$_A$ receptors (or partial agonist action at α1GABA$_A$ and/or α5GABA$_A$ receptors) attenuates rest posture induced by selective α3GABA$_A$ stimulation. These findings collectively raise the possibility that mild, but not more pronounced, sedative effects may be induced by α3GABA$_A$–selective agonists. Indirect support for this possibility comes from the observation that a novel α3GABA$_A$–selective agonist (NG2-73) recently was in clinical trials for treatment of insomnia (Wafford and Ebert 2008).

Taken together, the data from the present set of experiments provide further evidence that the behavioral effects of benzodiazepines in primates are likely mediated by different GABA$_A$ receptors that contain distinct α subunits. Accordingly, our studies highlight several hypotheses regarding benzodiazepine action. First, our findings suggest that α3GABA$_A$ receptors play an important role in benzodiazepine-induced anxiolysis. In addition, results from the present set of experiments also implicate α3GABA$_A$ receptors in the myorelaxant and, potentially, the mild sedative effects associated with benzodiazepines. Finally, our results are consistent with the idea that the hyperphagic, ataxic and more pronounced sedative effects of benzodiazepines require actions additional to those at α3GABA$_A$ receptors. These hypotheses should provide an important framework for studying the role of different GABA$_A$ receptor subtypes in the behavioral effects of benzodiazepine-type drugs, which in turn should help guide both the current clinical use of benzodiazepines as well as the development of improved therapeutic agents for treating anxiety and sleep disorders.

Acknowledgments

We acknowledge the assistance of Dr. Annemarie Duggan, Kristen Bano, and Shana Langer with these studies. We also thank Dr. Roger Spealman for comments on an earlier version of this manuscript. This work was supported by USPHS grants DA11792, RR00168, and a collaborative research agreement from Merck Research Laboratories with Dr. Rowlett as the Principal Investigator. Drs. Atack, Reynolds, and Dawson were employees of Merck Research Laboratories (Merck, Sharp & Dohme, Ltd.). Drs. Fischer and Platt had no financial relationship with Merck Research Laboratories. The agreement between Harvard Medical School and Merck Research Laboratories was a scientific collaboration, and did not involve studies that by the publication thereof would engender financial gain or loss from public disclosure.

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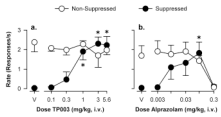

Figure 1.

Anti-conflict effects of TP003 (a) and alprazolam (b) in rhesus monkeys trained under a multiple schedule of food presentation (non-suppressed responding) and food + shock presentation (suppressed responding). Abscissae, cumulative intravenous dose of drug in mg/kg, i.v. Ordinates, response rate as responses per second. Each data point represents the mean (± S.E.M.) from three or four monkeys. Points above "V" represent data after vehicle administration. Asterisks represent significant differences relative to vehicle (Bonferroni t-tests, $p<0.05$).

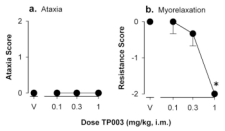

Figure 2.

Ataxia (a) and resistance (myorelaxation, b) scores of TP003 in squirrel monkeys as determined using quantitative observational procedures. Abscissae, dose of TP003 in mg/kg, i.m. Ordinates, assessment score as described in Materials and Methods section. Each data point represents the median and inter-quartile range from four monkeys. Points above "V" represent data after vehicle administration. Asterisks represent significant differences relative to vehicle (Dunn's Q statistic, $p<0.05$).

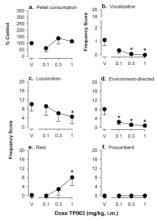

Figure 3.

Quantitative behavioral effects of TP003 in squirrel monkeys as determined in the observation procedure. Abscissae, dose of TP003 in mg/kg, i.m. Ordinates, pellet consumption as the mean number of sucrose pellets consumed expressed as percentage of baseline control (a) or frequency score as described in Materials and Methods section (b–f). Each data point represents the mean (± S.E.M.) from five monkeys. Points above “V” represent data after vehicle administration. Asterisks represent significant differences relative to vehicle (Bonferroni t-tests, $p<0.05$).

Table 1

Potencies of TP003, alprazolam, HZ-166 and zolpidem to alter suppressed and non-suppressed responding in the conflict procedure

Drug	Selectivity	Suppressed ED_{50} (95% CL)	Non-suppressed ED_{50} (95% CL)	Ratio[a]
TP003	α3	0.53 (0.48–1.1)	> 5.6	> 11 [b]
Alprazolam	α1,2,3,5	0.012 (0.0004–0.055)	0.12 (0.068–0.26)	10
HZ-166 [c]	α2,3	0.80 (0.17–2.0)	> 10	> 13 [d]
Zolpidem [e]	α1	– [f]	0.34 (0.13–4.2)	–

[a] Ratio= Non-suppressed ED_{50}/ Suppressed ED_{50}

[b] The highest dose tested (5.6 mg/kg) did not decrease rates of non-suppressed responding to < 50% in any monkey. To calculate the potency ratio, an ED_{50} of 5.6 was assigned.

[c] derived from Fischer et al. 2010

[d] the highest dose tested (10 mg/kg) did not decrease rates of non-suppressed responding to < 50% in any monkey. To calculate the potency ratio, an ED_{50} of 10 was assigned.

[e] derived from Rowlett et al. 2006

[f] could not be calculated

Table 2

Ataxic- and myorelaxant-like effects of TP003, alprazolam and zolpidem in the observation procedure. Median scores (±interquartile range) are from the maximally effective dose tested (shown in parenthesis).

Drug	Myorelaxation	Ataxia
Vehicle	0 (0 – 0)	0 (0 – 0)
TP003 (1 mg/kg)	−2 (−2 — −2) *	0 (0 – 0)
Alprazolam (1 mg/kg) [a]	−2 (−1.835 — −2) *	1.67 (1.67 – 1.835) *
Zolpidem (10 – 17.8 mg/kg) [a]	−1.835 (−1.17 — −2) *	2 (1.5 – 2) *

[a] derived from Licata et al. 2009

* Dunn's Q statistic, $p<0.05$

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Table 3

Effects of TP003, alprazolam and zolpidem on observational measures related to sedation. Frequency scores (±S.E.M.) are from the maximally effective dose tested (shown in parenthesis).

Drug	Pellet Consumption (% Control)	Locomotion	Environment-directed	Rest	Procumbent
Vehicle	100	10.1 ± 3.1	8.1 ± 2.6	0.3 ± 0.2	0 ± 0
TP003 (0.3 – 1)	137 ± 42	4.5 ± 3.1 *	0.9 ± 0.6 *	8.1 ± 3.6 *	0 ± 0
Alprazolam (0.3 – 1) [a]	255 ± 21 *	0.8 ± 0.3 *	0 ± 0 *	6.7 ± 3.6	16.1 ± 2.5 *
Zolpidem (10 – 17.8) [a,b]	292 ± 55*	1.2 ± 0.9 *	1 ± 0.8 *	3.4 ± 2.8	9.4 ± 4.0 *

[a] derived from Duke et al. 2006

[b] derived from Platt et al. 2002

* Bonferroni t test, $p<0.05$