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Pharmacological differences of GABAergic compounds: a pharmacodynamic characterization

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CHAPTER 7

The specificity of different selective and non-selective GABA_A (partial) agonists in healthy volunteers

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ABSTRACT

Different selective GABA_A-ligands are in development to treat anxiety and sleep disorders. The Central Nervous System (CNS) effects of various selective and non-selective (partial) GABA_A agonists were investigated, using several pharmacodynamic measurements. Comparison in selectivity is only reliable at equipotent doses for the intended clinical effect. For novel compounds, these data are not available, but selectivity may also be determined by comparing the effects on different CNS-parameters.

The full agonist lorazepam was compared to another full agonist midazolam, the α_1 -selective zolpidem and to three novel $\alpha_2,3$ -selective (partial) agonists, TPA023, MK-0343 and SL65.1498. All studies had placebo-controlled, double-blind, cross-over designs. Slopes of the linear relationships between Δ (change from baseline) body sway and Δ SPV, and between Δ VAS alertness and Δ SPV, were compared among compounds. Effect-relationships were also semi-quantitatively compared to the pharmacological characteristics of the compounds, estimated from in vitro literature data.

Slopes of both relationships did not differ clearly between lorazepam and midazolam. For SL65.1498, slopes were similar to those of lorazepam. In contrast, TPA023 and MK-0343 differed clearly from lorazepam, for both relationships. Compared to these compounds, zolpidem showed relatively high reductions in VAS alertness, although effect-relationships were not significantly different from those of lorazepam.

These results support the selectivity of some subtype-selective partial GABA_A agonists. The therapeutic relevance of these differences remains to be established. Comparisons of relative effect profiles can provide meaningful insights into the selectivity of different (partial) agonists, relatively independent of dose levels.

INTRODUCTION

Anxiety disorders are prevalent, enduring and often disabling [1]. Although the pathophysiology is poorly understood, the GABA_A receptor complex plays an important role in the modulation and mediation of pathologic anxiety states [2-5]. Benzodiazepines, which bind to the benzodiazepine GABA_A receptor, are highly effective therapeutic agents for the treatment of anxiety [1]. The introduction of the benzodiazepines in the 1960s was considered a significant improvement in the treatment of anxiety, and they became the drugs of choice based on their safety and fast onset of action. However, benzodiazepines also have undesirable side effects like sedation, balance problems, memory disturbance, tolerance development and abuse potential. Particularly in the elderly, these adverse effects are associated with higher incidences of falls [6,7] and cognitive impairment [8,9].

Benzodiazepines (BZs) bind to and act upon a subset of GABA_A receptors containing a pentameric composition of two β and two γ subunits in combination with one α 1-, α 2-, α 3- or α 5 subunit [10] that are formed like a rosette around a chloride channel. Three quarters of the GABA_A receptors possess a BZ binding site. The composition of the receptor is diverse in different brain regions and relatively few of the theoretically possible subunit combinations occur in vivo. This heterogeneity of GABA_A receptors in different neuro-anatomical localizations suggests discrete physiological functions for different receptor populations. Non-selective binding of benzodiazepines to these different GABA_A receptor populations might therefore produce distinct components of pharmacological response, including the adverse side effects.

In the 1990s, non-selective partial GABA_A agonists were introduced [11], which were thought to provide anxiolytic activity, based on the assumption that the receptor reserve for anxiolytic activity would be higher than that for other (undesired) pharmacological actions. For most compounds however, a pre-clinical non-sedating anxiolytic profile could not be translated in an improved therapeutic window [12-14].

In order to develop compounds that selectively modulate only certain GABA_A receptor subtypes, knock-in and -out mice models were used to elucidate the role of the different α subtypes. It was found that the α 2,3 subunits of the GABA_A receptor are responsible for anxiolysis and muscle relaxation [15-19], while the α 1 subunit is associated with sedation [19-22] and postural instability [23,24]. The α 5 subtype seems to be associated with memory and cognition [25-27]. These findings have stimulated the search for ligands for different GABA_A receptor subtypes with a higher therapeutic selectivity. This

is intended to result in an anxiolytic compound that is devoid of the unwanted effects of existing benzodiazepines. There are two options to attain this selectivity: selective affinity and selective efficacy for a certain subtype. The first option first resulted in sleep inducers with a higher affinity for the α_1 receptor subtype (e.g. zolpidem, zaleplon) [28]. More recently, compounds with a higher affinity for the anxiolytic (α_2 or α_3) GABA_A receptor subtypes have been developed [29-33]. Selective efficacy was the base for the development of L-838417 [21], SL65.1498 [20,24,34], Compound 4 [35], NSX [36] and TPA023 [31,33,37]. Selective efficacy can be achieved by (partial) agonistic activity at the $\alpha_2,3$ subtypes and less activity at the others (e.g. SL65.1498, MK-0343) or by (partial) agonistic activity at the $\alpha_2,3$ subtypes, combined with antagonistic activity at the α_1 and α_5 subtypes (e.g. TPA023). These compounds with selective efficacy have shown to be promising in pre-clinical studies, but this could so far not be confirmed in clinical trials [38]. At present, ocinaplon [39] and ELB-139, a GABA_A α_3 subtype-selective agonist [40,41], seem to be most advanced in their clinical development [38]. Unexpectedly, ocinaplon seems anxiolytic and non-sedating in patients despite a preclinical pharmacological profile that is relatively α_1 subtype selective [39].

Thus, it seems that animal models have only limited predictive value for subtype selectivity of GABA_A (partial) agonists in humans. However, healthy volunteers exhibit a range of Central Nervous System (CNS) responses to non-selective benzodiazepines [12,42-44], which may reflect an activation of different GABA_A receptor subtypes. If so, these CNS-responses may be appropriate biomarkers in healthy volunteers, which could be predictive for the clinical effects in patients.

This study was performed to explore some of the relationships between various CNS-effects and pharmacological properties of GABA_A agonists in healthy humans. We have investigated several subtype-selective and non-selective GABA_A receptor agonists using a validated CNS test battery, including a range of different CNS-functions that have all been shown to be very sensitive to non-selective GABA_A agonists (benzodiazepines), such as eye movements, body sway and Visual Analogue Scales (VAS) of alertness, contentedness and calmness [43,45,46]. It is not unreasonable to assume that the series of benzodiazepine effects reflect activation of different GABA_A receptor subtypes in different brain areas, although the nature of these relationships is unknown in human. Preclinical experiments and the effects of zolpidem suggest that subjective alertness in humans is related to α_1 -stimulation. Memory effects (which we did not test uniformly with all our compounds) seem to be related to α_5 -receptor subtypes [25]. The case is less clear for impairment of body sway,

which could be caused by muscle relaxation, reflect reduced attention or result from cerebellar effects. Reduction of saccadic peak velocity has been shown to be closely related to the anxiolytic potencies of benzodiazepines [42], and could thus hypothetically reflect $\alpha_2,3$ -activity. Saccadic peak velocity is also reduced during sedation [47]. None of these parameters are necessarily pure biomarkers for any specific effect or GABA_A subtype. Nonetheless, it is expected that compounds with comparable pharmacological characteristics will all cause similar changes in these different CNS-effects.

An important requirement for such a comparison of effect profiles is that doses should be therapeutically equipotent. Unfortunately, the clinical effects and hence the therapeutic doses of the new subtype selective compounds are often unknown in early phases of development. To bypass this problem, we looked at the profiles of different CNS-effects, by comparing the relations between effects on body sway (postural (in)stability) and visual analogue scales of alertness, relative to saccadic peak velocity (SPV). This approach produced graphs that illustrated the relative effect profiles compared to full benzodiazepines, and also allowed for formal statistical testing.

We compared the relative effect profiles of the non-selective GABA_A ligands lorazepam and midazolam, to those of the novel $\alpha_2,3$ selective (partial) agonists TPA023, SL65.1498 and MK-0343, which are in development as anxiolytics. The selective α_1 agonist zolpidem was also used in this analysis.

METHODS

The studies that are used for this article were all performed in healthy male volunteers who signed informed consent before medical screening and participation. All studies were approved by the Medical Ethics Review Board of Leiden University Medical Centre, and performed according to their standards.

Data from five studies with different compounds, all performed at the Centre for Human Drug Research, were used for analysis of this manuscript. All studies included the same pharmacodynamic CNS measurements that were measured frequently at different time points until 8 hours post dose.

In vitro affinity and efficacy values were derived from Investigator's Brochures and published data [20,28,37,48]. Although the efficacies were determined using whole-cell patch clamping, different cell types and different GABA_A receptor homologues were used. A formal comparison between the pharmacological in vitro and human in vivo characteristics was therefore not considered to be possible, and the results were only evaluated semi-quantitatively.

Treatments

The following GABA_A-ergic drugs were administered in the different studies: TPA023 1.5 mg (n=12), mk-0343 0.75 mg (n=12), SL65.1498 25 mg (n=20), zolpidem 10 mg (n=14), lorazepam 2 mg (two studies n=24), midazolam 0.1 mg/kg during 25 min constant infusion (two studies n=35). All treatments were administered as capsules to subjects in the fasted state, except for midazolam, which was given intravenously.

Therapeutically relevant doses of zolpidem, lorazepam and midazolam were used as positive controls in the studies. For the investigational treatments (TPA023, MK-0343 and SL65.1498), no clinical data are yet available, but the administered doses were expected to be anxiolytic, based on studies in animals and/or healthy volunteers.

Pharmacodynamic assessments

Saccadic Eye Movements

Saccadic eye movements were recorded using a computer-based system for data recording (Cambridge Electronics Design, Cambridge, UK), Nihon Kohden equipment for stimulus display, signal collection and amplification (Nihon Kohden Corporation, Tokyo, Japan), and disposable surface electrodes (Medicotest N 00 S, Olstykke, Denmark) [49]. Average values of latency (= reaction time), peak saccadic velocity and inaccuracy (difference between stimulus angle and corresponding saccade in %) were calculated for all artifact free saccades. Saccadic peak velocity has been validated as the most sensitive measure for the sedative effects of benzodiazepines [43,44]. Saccadic peak velocity (SPV) has also been shown to be closely related to the anxiolytic properties of benzodiazepines [42].

Visual Analogue Scale

Visual analogue scales as originally described by Norris [50] were previously used to quantify subjective effects of benzodiazepines [44]. From the set of sixteen scales three composite factors were derived as described by Bond and Lader [51], corresponding to alertness, mood and calmness. These factors were used to quantify subjective drug effects.

Body Sway

Body sway was measured with an apparatus similar to the Wright ataxiometer [52], which integrates the amplitude of unidirectional

body movement transferred through a string attached to the subject's waist. Two-minute measurements were made in the antero-posterior direction with eyes closed, with subjects standing comfortably on a firm surface with their feet slightly apart. Body sway is a measure of postural stability that has previously been shown to be sensitive to benzodiazepines [12].

Statistical analyses

Body sway values were log-transformed as data were clearly skewed. As the maximum effect of zolpidem and SL65.1498 on SPV, body sway and VAS alertness was much smaller than the effect of lorazepam, only the data outside the effect peak of lorazepam (from 0-55 min and 300-355 min) were used for the analysis. Due to the short half-life of zolpidem, measurements of 6 hours post-dose, which are a long time after peak effect, are not included in the analysis for this compound. These data points showed baseline levels of effects and would otherwise incorrectly influence the analysis.

For the analysis a regression of change from baseline of body sway or VAS alertness on change from baseline of SPV on the individual data was performed with a mixed model with treatment as fixed factor and SPV change from baseline and intercept as random factors. The estimate of the slopes of the linear relations between change from baseline in body sway and SPV, and between VAS alertness and SPV, were compared between the subtype-selective GABA_A agonists and the benzodiazepines. The estimates of the slopes, their estimated difference and the p-values were tabulated.

All calculations were performed using sas for Windows V9.1.2 (sas Institute, Inc., Cary, NC, USA).

RESULTS

Relation between SPV and Body Sway

Figure 1 shows the averages of all subjects of SPV and body sway per study on the different time points. Table 2 shows the estimated average slopes for the different compounds and the comparison with lorazepam. TPA023 and MK-0343 showed a different effect on body sway relative to SPV when compared to lorazepam (95%CI: 2.52/ 4.26 and 2.65/4.36 respectively). The estimated slopes for SL65.1498 and zolpidem did not differ from lorazepam (95%CI: -0.09/1.95 and -2.21/0.91, respectively). Midazolam also showed a different effect relationship compared to that of lorazepam ($0.34 \cdot 10^{-3} / 2.22 \cdot 10^{-3}$). This was due to a loop in the SPV-body sway-relationship for midazolam that was

not observed for lorazepam. The upper part of the loop could not be described, because for safety reasons body sway was first measured only 36 minutes after the start of the midazolam infusions.

Relation between SPV and VAS Alertness

Figure 2 shows the averages of all subjects of SPV and VAS alertness per study on the different time points. Table 3 shows the estimated average SPV/VAS alertness slopes for the different compounds and the comparison with lorazepam. Both TPA023 and MK-0343 showed a different effect on VAS alertness relative to SPV when compared to lorazepam (95%CI: -0.18/-0.075 and -0.11/-0.011, respectively), while estimates of slopes for midazolam (95%CI: -0.11/0.012), SL65.1498 (95%CI: -0.11/0.024) and zolpidem (95%CI: -0.11/0.13) did not differ from lorazepam.

Relationships with in vitro pharmacological characteristics

In vitro affinities and relative efficacy values of the different compounds as determined from the literature are presented in Table 1. No simple relationships could be identified between the pharmacological characteristics and individual pharmacodynamic properties. TPA023 and MK-0343, which both showed the most specific effect relationship profiles, also had the lowest efficacies in vitro for the α_1 -receptor subtype. Zolpidem and SL65.1498, whose effect relationships were much more benzodiazepine-like, had much higher α_1 -efficacy values than any other compound.

DISCUSSION

This analysis was performed to compare the Central Nervous System (CNS) effects of a range of GABA_A ligands with different pharmacological binding and efficacy profiles, to get an impression of their selectivity in comparison with non-selective full benzodiazepine agonists in healthy volunteers. Different CNS-effects were chosen, that have been shown to be sensitive to benzodiazepines, and which reflect different types of effects that could be related to distinct GABA_A subtypes –although it was impossible to determine the nature of these relationships a priori. Based on preclinical data, it was hypothesized that visual analogue scales of subjective alertness could reflect α_1 -receptor subtype activity [19-22]. Memory effects could be related to α_5 -receptor subtypes [25-27], but this could not be addressed

since we did not employ uniform memory tests in each individual study. The associations were less evident for saccadic peak velocity, which could be associated with anxiolysis [42], which is hypothetically related to $\alpha_{2,3}$ -receptor subtypes [15-18], but also with sedation [47], associated with α_1 -receptor subtypes. Body sway (postural instability) could have a potential association with both α_1 -receptor subtypes (as a manifestation of sedation) [23,24] or $\alpha_{2,3}$ subtypes (muscle relaxation) [15,16]. Thus, it was impossible to formulate unequivocal a priori hypotheses, regarding the associations between these CNS-effects, specific GABA_A receptor subtypes and the drugs' pharmacological properties. But even using an empirical approach, it was reasonable to assume that these associations would be similar for compounds with comparable pharmacological characteristics. Conversely, if similarity could be confirmed among benzodiazepines, differences between non-selective and novel selective compounds could be viewed as indicators for their pharmacological disparities.

The comparisons showed that intravenous midazolam did not differ substantially from oral lorazepam, except for different time-relationships between body sway and spv effects. In contrast, the novel partial $\alpha_{2,3}$ -receptor subtype-selective GABA_A agonists TPA023 and MK-0343 exhibited relatively reduced subjective sedation and postural imbalance. On sedation, the opposite was found for zolpidem, a relatively α_1 -selective non-benzodiazepine hypnotic, although the difference with lorazepam failed to reach statistical significance. SL65.1498 had much smaller effects than any of the other compounds, but across this range the slopes for the different effects were similar to those of the benzodiazepines. The average graphs (Figures 1 and 2) give a clear overview of differences and similarities among the compounds.

These results differed somewhat from preclinical findings. The three functional selective GABA_A ligands TPA023, MK-0343 and SL65.1498 had comparable effects in animal studies. They all showed anxiolytic effects at doses that were not sedative. TPA023 did not show any sedation in animal studies [31,37], which is probably due to the antagonistic effects at the α_1 subtype. Despite similar effect profiles in animal models, the three compounds displayed different relations between effects in healthy volunteers. Thus, behavioural animal models did not accurately predict the effects in healthy volunteers. A similar conclusion was reached in an earlier study, where the partial non-selective GABA_A agonist bretazenil, which was predicted to be a 'non-sedating anxiolytic' in animal models, was found to be just sedative as diazepam in healthy volunteers [12].

Attempts were made to relate the differences in relative effect relationships to the pharmacological properties of the compounds. Only TPA023 and MK-0343 were examined using the same oocyte-clamp studies. It may be difficult to compare the in vitro

pharmacological efficacies from different studies and laboratories. However, with this caveat there seemed to be a partial agreement between the observed differences in effect relationships in our studies, and the pharmacological receptor efficacies found *in vitro*. Table 1 shows that the absolute magnitudes of the efficacies on the α_1 -receptor subtypes runs parallel to the selectivity of the relationships that we observed in humans. In preclinical studies, α_1 -efficacy is often expressed relative to the efficacy on $\alpha_{2,3}$ -receptor subtypes, to determine whether an anxiolytic GABA_A agonist is relatively non-sedative. However, SL65.1498 showed a relative low $\alpha_1/\alpha_{2,3}$ -ratio (0.39 and 0.54) compared to that of Mk-0343 (0.78 and 0.4), whereas their effect profiles were quite different. This suggests that compounds should have a very low absolute α_1 -efficacy to be relatively non-sedative. Despite its relative selectivity, the α_1 -efficacy of SL65.1498 may be too high in absolute terms to allow an effect differentiation for this compound. Behavioural animal studies, in which rats were trained to discriminate between chlordiazepoxide or zolpidem, have also shown that SL65.1498 generalized to both the triazolam and zolpidem cue [36]. This is another indication for a significant efficacy at the α_1 subtype, which is shared by the reference drugs. This could explain why SL65.1498 behaved much like a non-selective (partial or low-dosed) GABA_A agonist in healthy volunteers. These comparisons suggest that *in vitro* receptor efficacy should not only be selective for a certain desired subtype, but also be sufficiently low to avoid undesired clinical effects. The same reasoning could apply to the α_2, α_3 -efficacies needed for anxiolytic effects, but this cannot be determined without detailed information about the anxiolytic potencies of these novel agents.

An important prerequisite for our approach was that non-selective benzodiazepines showed similar relative effect relationships. This condition was met with some constraints. The profiles for midazolam showed a loop for both the body sway/SPV- and VAS/SPV-relationships (Figures 1 and 2), which was not the case for lorazepam. For the VAS/SPV-relationships, this did not lead to differences in the slopes of the curves. For the body sway/SPV-relationship, the initial (upper) part of the loop could not be described, because in these experiments body sway was only first measured after 36 minutes, to avoid falls during the midazolam infusion phase (in contrast to SPV and VAS alertness). The incomplete loop was tilted to the lower part of the curve, which led to a statistically significantly different slope compared to lorazepam. We do not believe this represents a real difference in selectivity between the compounds. Rather, the loop for midazolam seems to be a consequence of intravenous drug administration. The rapid rise in plasma concentrations appears to reveal different equilibration half lives for the various CNS-effects, which seem slower for SPV than for body sway or VAS alertness. These differences did not become apparent with the

slow rise in plasma concentrations after oral dosing of lorazepam. This may also explain the smaller loops observed for zolpidem, which is absorbed very rapidly after oral administration [53] (Figures 1 and 2).

To test the validity of the relative effect profiles as predictors of clinical efficacy, the α_1 -selective non-benzodiazepine zolpidem was included in the analyses. Zolpidem has been developed as a selective hypnotic with higher affinity for the α_1 subtype, which is believed to be associated with sedation [19-22]. The effect on VAS alertness seemed to be more substantial than the effects on SPV, which was the opposite of the results for compounds with relatively little α_1 -efficacy (Figure 2). This is in line with expectations, although the contrast with lorazepam failed to reach statistical significance. The body sway/SPV relationship did not differ from that of lorazepam. The equivocal distinctions between zolpidem and the benzodiazepines are a reflection of the literature. Some studies have suggested that zolpidem is a relatively weak anxiolytic [54], which would agree with a relatively small SPV-reduction in proportion to the sedative effects as suggested by Figure 2. On the other hand, several other studies have been unable to show consistent clinical differences between zolpidem and non-selective benzodiazepines [55,56]. It is also known that zolpidem causes postural instability [23], which is an argument that body sway increases are related with α_1 - [24] rather than α_2 -activity. The major clinical benefit of zolpidem as a sleep-onset hypnotic with relatively few side effects after awakening seems to be the result of its rapid action with a quick absorption and short elimination half-life, rather than its α_1 -subtype selectivity. Although we found few differences between zolpidem and benzodiazepines, it could still be selective. Zolpidem did not show any effects on memory tests that we performed with this compound [53], whereas memory was consistently affected with lorazepam in other studies by our group [57,58]. A direct comparison of the memory effects of the different compounds could have provided interesting information on their relative α_5 -efficacies, but unfortunately different methods of memory testing were used in the various studies.

It could be argued that a comparison of the different compounds is precluded by a lack of equipotency. We have taken several precautions that should allow a reasonable comparison. First, doses of the investigational compounds were all predicted to be in the therapeutic range based on animal studies, and therapeutically relevant doses were used for the registered compounds. Plasma levels that were measured during the individual studies in healthy volunteers were confirmed to be similar to the anxiolytic and non-sedative levels in animals. Based on the results of our studies, it cannot be excluded that the dose of SL65.1498 was low in comparison to the other compounds. This dose was mainly chosen to avoid (sedative) side effects that were found with higher doses in early healthy volunteer studies. However, we

particularly looked at the relations of different parameters relative to SPV reduction, which has been shown to be closely associated with anxiolytic effects of benzodiazepines [42]. These measures should have formed a sound basis for a comparison of drugs at doses that are not exactly equipotent.

SL65.1498 had very limited effects on any pharmacodynamic parameter. Therefore, it can be expected that this compound will not only be devoid of sedative effects, but SL65.1498 may also not be a very potent anxiolytic -at least at a dose of 25 mg. In contrast, TPA023 and to a lesser extent MK-0343 showed selective effects on SPV (which has been related to the anxiolytic potencies of benzodiazepines [42]) without many other CNS-effects. These drugs could be expected to be anxiolytic with reduced sedative or postural side effects. Preliminary results of a fear-potentiated startle (FPS) paradigm in healthy subjects support the anxiolytic efficacy of single dose TPA023 2.0 mg (MSD, unpublished data). In that study, startle amplitude during threat conditions was significantly reduced when subjects received TPA023 2.0 mg compared to placebo ($p < 0.0035$). So far, clinical data on the effects of these drugs in patients with an anxiety disorder are limited. TPA023 and SL65.1498 have been studied in anxiety patients, but the results have not been published. For undisclosed reasons, the development of all agents reported in this article does not seem to have continued for anxiety disorders. The results of the FPS trial with TPA023 support the findings of our exploratory analysis, but the predictive value of our studies in healthy volunteers will require corroboration in anxiety patients, where the relationships may be different.

In conclusion, the selective $\alpha_{2,3}$ -agonists TPA023 and MK-0343 cause relatively little subjective sedation and postural imbalance, compared to full benzodiazepine agonists. This corresponds to their α_1 -receptor efficacy in vitro. SL65.1498 showed a much higher α_1 -efficacy and was much less selective in healthy volunteers. The effects on body sway of the α_1 -selective compound zolpidem were very similar to those of non-selective benzodiazepines, whereas the sedative effects of zolpidem seemed more pronounced. This indicates that many of the inadvertent effects of GABAergic anxiolytics are due to α_1 -activation, and some α_1 -antagonism may be needed to avoid them altogether. These results provide support for the selectivity of some partial subtype-selective GABA-agonists, compared to a full-agonist benzodiazepine. Comparisons of pharmacodynamic profiles between different compounds, by analysis of the relative effect relationships, can provide meaningful insights into the selectivity of different (partial) $\alpha_{2,3}$ agonists, which is relatively independent of the potencies of the doses. In the future, when more therapeutic results of clinical trials are available, the significance and predictive value of these analyses should be substantiated further.

Table 1 Comparison of benzodiazepine-binding properties of recombinant GABA_A receptors with different subtypes

Compound	α 1		α 2		α 3		α 5	
	K _i (nM)	Efficacy ° (%)	K _i (nM)	Efficacy ° (%)	K _i (nM)	Efficacy ° (%)	K _i (nM)	Efficacy ° (%)
TPA023 ^a (a)	0.27	0	0.31	11	0.19	21	0.41	5
MK-0343 ^b (b)	-	18	-	23	-	45	-	18
SL65.1498 ^c (c)	17	45	73	115	80	83	215	48
Zolpidem	20 (d)	75 §(e)	400 (d)	78 §(e)	400 (d)	80 §(e)	5000 (d)	9 §(e)

° Relative efficacy is defined as the extent of the potentiation of GABA_A EC20-equivalent current produced by the compound compared to that produced by a non-selective full-agonist (chlordiazepoxide/diazepam). a [37], b [58], c [20], d [28], e [48] * Mean values of 3 experiments in *Xenopus* oocytes with human recombinant αβγ2 receptors efficacy relative to chlordiazepoxide # Mean values of 3 experiments in HEK293 cells with recombinant rat receptors αβγ2 efficacy relative to chlordiazepoxide § Mean values of 3 experiments in *Xenopus* oocytes with human recombinant αβγ2 receptor efficacy relative to diazepam.

Table 2 Estimated slopes of relationship between log body sway (in log mm) and saccadic peak velocity (SPV in sec·deg⁻¹) for each treatment and the difference in slope between lorazepam and the treatment.

Treatment	Estimate of treatment (logmm·sec·deg ⁻¹)	Estimate of Lorazepam (logmm·sec·deg ⁻¹)	Estimate of difference Lorazepam-treatment (logmm·sec·deg ⁻¹)	95% CI	
Midazolam	-3.2·10 ⁻³	-4.5·10 ⁻³	1.3·10 ⁻³	0.34·10 ⁻³	2.22·10 ⁻³
SL65.1498 ^c	-1.5·10 ⁻³	-2.5·10 ⁻³	0.93·10 ⁻³	-0.09·10 ⁻³	1.95·10 ⁻³
TPA023	-1.1·10 ⁻³	-4.5·10 ⁻³	3.4·10 ⁻³	2.52·10 ⁻³	4.26·10 ⁻³
MK-0343	-0.97·10 ⁻³	-4.5·10 ⁻³	3.5·10 ⁻³	2.65·10 ⁻³	4.36·10 ⁻³
Zolpidem ^a	-3.1·10 ⁻³	-2.5·10 ⁻³	-0.65·10 ⁻³	-2.21·10 ⁻³	0.91·10 ⁻³

* Lorazepam data between 55–300 min were not used for analysis

Table 3 Estimated slopes of relationship between VAS alertness (in mm) and saccadic peak velocity (SPV in sec·deg⁻¹) for each treatment and the difference in slope between lorazepam and the treatment

Treatment	Estimate of treatment (mm·sec·deg ⁻¹)	Estimate of Lorazepam (mm·sec·deg ⁻¹)	Estimate of difference Lorazepam-treatment (mm·sec·deg ⁻¹)	95% CI	
Midazolam	0.16	0.21	-0.048	-0.11	0.012
SL65.1498 ^c	0.090	0.13	-0.042	-0.11	0.024
TPA023	0.085	0.21	-0.13	-0.18	-0.075
MK-0343	0.15	0.21	-0.062	-0.11	-0.011
Zolpidem ^a	0.14	0.13	0.0082	-0.11	0.13

* Lorazepam data between 55–300 min were not used for analysis

Figure 1 Graphs show mean change of baseline of body sway versus saccadic peak velocity (SPV). Closed circles show lorazepam and open squares reflect comparing treatment. Dashed line for midazolam is drawn to indicate the missing points for body sway measurement (36 min.) during the midazolam infusion phase.

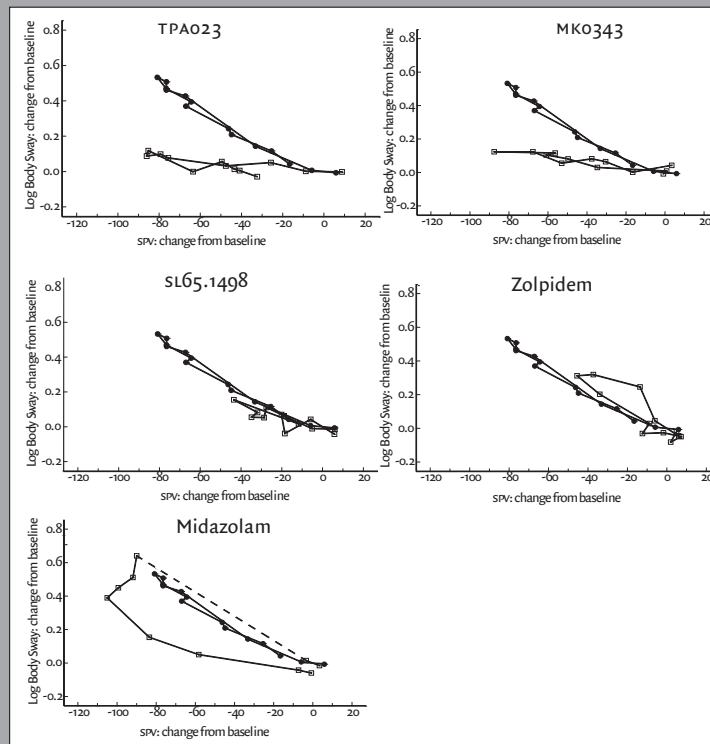
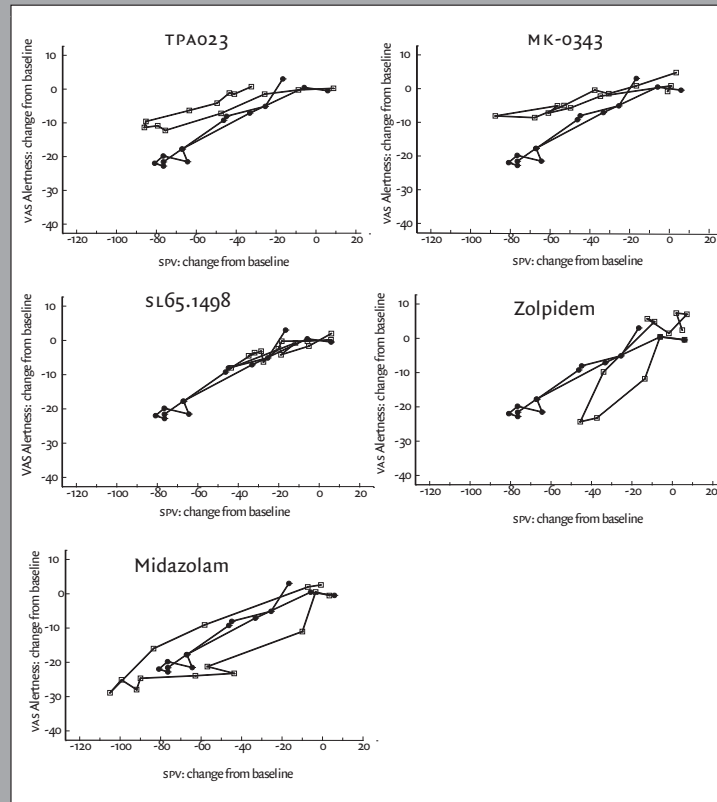


Figure 2 Graphs show mean change of baseline of VAS alertness versus saccadic peak velocity (SPV). Closed circles show lorazepam and open squares reflect comparing treatment.



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