Human pharmacology of current and novel gaba(a)-ergic treatments for anxiety

Chen, X.

VII

PHARMACODYNAMIC RESPONSE PROFILES OF ANXIOLYTIC AND SEDATIVE DRUGS

British Journal of Clinical Pharmacology 2017; 83(5): 1028-1038.

Xia chen[1,2], Freerk Broeyer[2], Marieke de Kam[2], Joke Baas[3], Adam Cohen[2], Joop van Gerven[2]

1. Phase I Unit, Clinical Pharmacology Research Center, Peking Union Medical College Hospital, Beijing, China | 2. Centre for Human Drug Research, Leiden, The Netherlands | 3. Department of Experimental Psychology, Faculty of Social Sciences, Utrecht University, The Netherlands

ABSTRACT

Introduction: Centrally-acting acutely anxiolytic drugs, like benzodiazepines, barbiturates and gabapentinoids, affect various central nervous system (CNS) functions, which reflect not only their anxiolytic effects but also neuropsychological side-effects. To validate the pharmacodynamic biomarkers for GABAergic anxiolytics, this study determined the pharmacodynamics of two anxiolytics and a non-anxiolytic control and linked them to their anxiolytic and sedative effects, during an anxiety-challenge study day. **Methods:** Twenty healthy volunteers were randomized in this placebo-controlled, double-blind, four-way cross-over study with single-dose alprazolam (1 mg), diphenhydramine (50 mg), pregabalin (200 mg) or placebo. The Neurocart® was used in-between repeated fear-potentiated-startle assessments. Thus the potential influence of anxiety on CNS pharmacodynamic markers could be examined. **Results:** Compared to placebo, $VAS_{calmness}$ increased with alprazolam (2.0 mm) and pregabalin (2.5 mm) but not with diphenhydramine. Saccadic-peak-velocity (SPV) declined after alprazolam (-57 deg/sec) and pregabalin (-28 deg/sec), more than with diphenhydramine (-14 deg/sec); so did smooth-pursuit. The average responses of SPV and smooth-pursuit were significantly correlated with the drug-induced increases in $VAS_{calmness}$. The SPV-relative responses of $VAS_{alertness}$, body-sway and adaptive-tracking also differed among alprazolam, pregabalin, and diphenhydramine. **Conclusions:** Compared with the antihistaminergic sedative diphenhydramine, alprazolam and pregabalin caused larger SPV reduction, which was correlated with simultaneous improvement of subjective calmness, during a study day in which anxiety was stimulated repeatedly. The different effect profiles of the three drugs are in line with their pharmacological distinctions. These findings corroborate the profiling of CNS effects to demonstrate pharmacological selectivity, and further support SPV as biomarker for anxiolysis involving GABAergic neurons. The study also supports the use of prolonged mild threat to demonstrate anxiolytic effects in healthy volunteers.

INTRODUCTION

Centrally-acting acute anxiolytic drugs, like benzodiazepines, barbiturates and gabapentinoids, have an impact on a range of central nervous system (CNS) functions, which reflect not only their anxiolytic effects but also side effects like sedation, postural instability and visuomotor and memory impairment [1]. It would be useful to identify the CNS activities for those compounds that are more closely linked to reduction of anxiety than to general CNS depression.

Pharmacodynamic (PD) approaches have been increasingly employed in early human pharmacology studies to obtain in vivo pharmacological information of different drugs acting on the central nervous system and of the systems with which the drugs interact. The general aim of these methodology is to obtain information about the pharmacological characteristics of a drug (such as blood-brain barrier penetration, target engagement and mechanistically meaningful activity), which underlie its therapeutic effects [2-4]. The use of appropriate biomarkers may be especially useful for anxiety disorders, where therapeutic exploratory studies in patients can be difficult to achieve a clinically meaningful end-point due to the nature of subjective assessments, the relatively large size and probability of placebo effect, and other ethical or practical issues [5,6]. Of no doubt, a validated biomarker in early human pharmacology studies would serve as a useful tool for the development of new therapeutic anxiolytics.

It has been well established that benzodiazepines (BZDs) exert their pharmacological effects through positive allosteric modulation of the GABA-A receptors. Recent years, the experiments on GABA-A receptor subtype-gene knock-out mouse lines has greatly facilitated the identification of GABA-A receptor subtypes that mediates BZDs-induced sedation (α_1 GABA-A receptors), anxiolysis (α_2 and α_3 GABA-A receptors), or memory impairment (α_5 GABA-A receptors) [7-9]. To address the effects of BZDs in human pharmacological studies, a collection of pharmacodynamic measurements were employed and evaluated for their pharmacokinetic/pharmacodynamic relationship with BZDs, which include objective measures such as electroencephalography, semi-subjective measures such as psychomotor performance, and subjective measures such as mood/sedation scales [10-13]. Despite of the acceptable sensitivity and the observed exposure-response relationship of these PD measurements for the effects of BZDs, as well as the potential involvement of eye movement in anxiety disorder and related neuropsychiatric disturbance, increasing attention has been paid to evaluate the relevance of these PD parameters to the pharmacological effects of established or novel anxiolytic drugs. The exact clinical relevance of quantitative electroencephalogram (EEG), for example, to the anxiolytic, anticonvulsant, sedative and hypnotic actions of benzodiazepines, have not yet clearly been elucidated [14].

The Centre for Human Drug Research (CHDR) (Leiden, The Netherlands) has developed a Neurocart battery of validated computerized tests for the assessments of various CNS functions. These tests have been shown to be sensitive to various aspects of sedation [15] and have been used in early studies of psychoactive drugs as pharmacodynamic biomarkers for postural (in)stability (body sway test), eye-hand cooperation (adaptive tracking test), subjective feelings of alertness, mood and calmness (visual analogue scale [VAS] Bond & Lader), and for neurophysiologic functions (saccadic eye movement and smooth pursuit eye movement tests) [6]. Our previous studies showed that the Neurocart battery presents distinct pharmacodynamic response-patterns to different subtype-selective partial GABA-A agonists and non-selective benzodiazepine anxiolytics [16-19], which may imply potential GABA-A subtype specificity of these PD markers. Normally, this test battery does not provide any clear information about the specific anxiolytic properties of drugs, as measured by $VAS_{calmness}$. Benzodiazepines or selective serotonin reuptake inhibitor (SSRIs), for instance, don't cause consistently significant increases of subjective calmness in healthy volunteers, when the measurement was performed in stress-free experimental settings [5,6]. Such findings can be true for SSRIs that have a slow onset of action and can even worsen anxiety symptoms during initial treatment [20], but is not expected for fast-acting anxiolytic drugs like benzodiazepines [21]. We therefore combined the Neurocart test battery with a modified fear-potentiated-startle (FPS) paradigm [22]. In this way, we could compare our more general CNS test battery with a specific anxiety test, which in some studies [23-24], but not all [25], has been shown to be sensitive to anxiolytic drugs. To this end, we administered two sedating anxiolytic drugs (alprazolam and pregabalin) and a sedating non-anxiolytic (diphenhydramine) at therapeutic doses to healthy volunteers.

METHODS

ETHICS

The study was approved by the Medical Ethics Review Board of Leiden University Medical Centre (LUMC), and was conducted according to the principles of the Helsinki Declaration and the International Conference on Harmonization/Good Clinical Practice (ICH/GCP).

DESIGN

This was a single-center, randomized, placebo-controlled, four-way crossover, double-blind study conducted in twenty healthy subjects. The scheme of this study included a screening period of maximally 14 days, four treatment periods separated by three washout periods of at least 3 days, and a telephone follow-up.

SUBJECTS

Ten men and ten women, aged between 18 and 40 years, with a BMI between 18 and 30 kg/m^2, without any clinically significant abnormalities, were recruited. All volunteers provided written inform consent. Their eligibilities were evaluated before being randomized into the study. Subjects were instructed not to use alcoholic beverages from 24 hours before admission until the next morning of each study day. No xanthine or tobacco containing products were allowed from 22:00 in the evening before each study day and during stay in the research unit. They were asked to keep a normal day/night pattern from two weeks before the first study day until the last study day.

SAMPLE SIZE DETERMINATION

As was shown in Grillon et al [23], the mean effect of the THREAT-SAFE difference between unpredictable threat and a neutral context seen under placebo was about 15 μV ± 8.5 μV whereas the effect under 1 mg alprazolam was around 5 μV ± 8.5 μV (mean ± standard deviation). This leads to an alprazolam effect of 10 μV over placebo. Given that the within patient variability is normally not substantially greater than the between patient variability a residual standard deviation of 10 μV was assumed. Based on these assumptions, a sample size of 16 subjects was obtained to ensure a power of at least 80% with a two-sided alpha level of 5%. For the Neurocart end points, using data from previous studies [17-19], the same sample size of 16 was determined to have equal to or greater than 80% power to detect the mean differences of 1.244 in VAS alertness and 20.577 in saccadic peak velocity (SPV), respectively assuming standard deviations of 1.663 (VAS alertness) and 27.429 (SPV) between placebo and lorazepam 2 mg using a paired t-test with a 0.050 two-sided significance level. Considering the possibility of drop-out and the sample should be a multiple of four (to keep the study design balanced the sample size), a total sample size of 20 subjects was finally decided for the study.

TREATMENTS

The study treatments were assigned according to a randomization schedule, which consisted of five blocks of the fully balanced 4*4 William Latin Squares. Each subject received single oral dose of over-capsulated pregabalin 200 mg, alprazolam 1 mg, diphenhydramine 50 mg or matching placebo in a fasted state at about 8 to 9 AM on each treatment period.

SAFETY

Adverse events, electrocardiograms (ECGs) and vital signs, as well as safety laboratory assays were frequently evaluated during the study. Twelve-Lead ECG recording was made using Nihon Kohden Cardiofax with Ecaps 12 software devices (Nihon Kohden, Tokyo, Japan). Vital signs (pulse rate and blood pressure) were taken using a Nihon-Kohden BSM-1101K monitor or a Colin Pressmate BP 8800. All blood pressure, pulse rate, and ECG recordings were done after subject was resting in a supine position for at least 5 minutes. Safety laboratory tests on blood or urine samples were performed in the Central Clinical Laboratories of LUMC.

PHARMACOKINETIC MEASUREMENTS

For the determination of drug concentrations, two venous blood samples of 5 and 2 ml were collected into ice-bathed Li-Hep tubes (Becton and Dickinson 367684 & 368200, respectively) within 0.5 hour pre-dose and at 0.5, 1.25, 1.75, 2.25, 3, 4, 6, and 8 hours post-dose. The samples were centrifuged (2000G, 15 min, 4°C). The obtained plasma was transferred into two polypropylene Sarstedt 2 ml tubes and stored at -20°C until analysis.

Plasma pregabalin concentrations were determined at AAI Pharma GmbH & Co KG, Neu-Ulm, Germany, using LC-MS/MS on a Finnigan LCQ system. A Phenomenex Gemini (50 x 3.0 mm i.d., 5µm) was used as the HPLC column. The quantification range was from 1.00 to 1000 µg/L. The intra- and inter-assay variability was 2.1-10.5% and 0.9-6.6%, respectively. Plasma alprazolam and diphenhydramine concentrations were determined at the pharmacy of the Groningen University Medical Centre, Groningen, the Netherlands, using LC-MS/MS. All experiments were performed on a ThermoFisher (San Jose, USA) triple quadrupole LC-MS/MS with a Finnigan™ Surveyor® LC pump and a Finnigan™ Surveyor® autosampler which was set at 20 °C. Lower limit of Quantification (LLOQ) was 1.00 µg/L for alprazolam and 5.00 µg/L for diphenhydramine, respectively. Intra- and inter- assay variability were 2.1-7.2% and 0.0-3.3%, respectively, for alprazolam and 2.0-3.3% and 0.0-2.0%, respectively for diphenhydramine.

PHARMACODYNAMIC MEASUREMENTS

A training session of the pharmacodynamic tests (i.e. the Neurocart battery and the FPS paradigm) was performed during the screening. The purpose was to familiarize the subjects with the tests and prevent potential learning effect. In each study period, the FPS paradigm was carried out around 1 hour after dosing; while the Neurocart battery was assessed at pre-dose and 0.5, 1.25, 1.75, 2.25, 3, 4, 6, and 8 hours post-dose in the following sequence of tests: body sway, VAS Bond & Lader, saccadic eye movements, smooth pursuit eye movements, and

adaptive tracking. At each assessment, one subject was assigned to a quiet room with ambient illumination.

Pharmaco- electroencephalograph (EEG) approach is currently widely used, and the empirical relation between this measure and other agonist effects of benzodiazepines has been reported. However, the main purpose of this study was to compare the sensitivity and specificity of the Neurocart PD measurements versus those of the FPS measurements to the effects of sedating, hypnotic, and anxiolytic drugs. As the flowcharts of the study days were already quite busy with the combination of the non-EEG PD tests and the FPS paradigm, and the device used for generation of electronic shocks in the FPS paradigm may interfere with the pharmaco-EEG measurements, the EEG measures were omitted from the study design for the sake of smooth operation.

BODY SWAY

Body sway was measured with an apparatus similar to the Wright ataxiameter [26], which integrates the amplitude of unidirectional body sway. The measurements were made in the antero-posterior direction with eyes closed for 2 minutes. The subject was asked to stand comfortably on a floor with his/her feet slightly apart. Body sway measures postural (in)stability. It has demonstrated considerable sensitivity to the effect of benzodiazepines [27].

VISUAL ANALOGUE SCALES OF BOND & LADER (VAS B&L)

Visual analogue scales, as originally described by Norris [28], were presented on a computer screen. Three composite factors were derived from the sixteen items, corresponding to alertness, mood and calmness, respectively. These factors quantify subjective feelings and have been extensively used to delineate subjective effects of a variety of sedative agents [6].

SACCADIC EYE MOVEMENTS

Saccadic eye movements were evaluated using a computer-based system composed of 1) stimulus display and signal collection (Nihon Kohden Corporation, Tokyo, Japan), 2) signal amplification (Grass-Telefactor, An Astro-Med, Inc. Product Group, Braintree, USA), 3) data recording (Cambridge Electronics Design, Cambridge, UK), 4) disposable silver-silver chloride electrodes (Medicotest N-00-S, Olstykke, Denmark), as well as 5) the sampling and analysis scripts developed by CHDR (Leiden, the Netherlands). The parameters of this test were the average values of saccadic peak velocity (SPV, degree/msec), reaction time (msec) and inaccuracy (%) of all artefact-free saccades that were calculated on each session. Saccadic peak velocity appears to be the most sensitive measure for the sedative effect of benzodiazepines [6] and has been found to be a promising biomarker for the anxiolytic component of benzodiazepines and some newly developed compounds with potential anxiolytic effect [16-19].

SMOOTH PURSUIT EYE MOVEMENTS

The same system as used for saccadic eye movements was also used for measurement of smooth pursuit. For smooth pursuit eye movements, the target moved sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, by step of 0.1 Hz. The amplitude of target displacement corresponded to 22.5 degrees eyeball rotation to both sides. Four cycles were recorded for each stimulus frequency. The method has been validated at the CHDR by van Steveninck *et al.* [29] based on the work of Bittencourt *et al.* [30] and the original description of Baloh *et al.* [31]. The time in which the eyes were in smooth pursuit of the target were calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies were used as the parameter.

ADAPTIVE TRACKING

The adaptive tracking test was performed as originally described by Borland and Nicholson [32], using customised equipment and software (Hobbs, 2004, Hertfordshire, UK). After a 0.5-minute run-in time without data-recording, the average performance over the rest 3.0 minutes was scored and was used as the test parameter. Adaptive tracking is a pursuit-tracking task. The subject was required to operate a joystick and try to keep a dot inside a circle moving randomly on the computer screen. If he/she succeeded, the speed of the moving circle increases, and vice versa.

FEAR POTENTIATED STARTLE (FPS) PARADIGM

The FPS paradigm is extensively described elsewhere [22]. In brief, the test contained three contexts, which differed in the possibility of electronic shocks signaled by a computer displayed verbal instruction: 'No shock' for the Neutral (N) context, 'Shock only during cue' for the Predictable (P) context, and 'Shock at any time' for the Unpredictable (U) context. Duration of each context was 90-100 sec, during which six startle probes were administered together with the assessment of startle response. Intervals between startle probes varied between 12 and 18 sec (16 sec on average). The FPS session consisted of two blocks with the following orders of contexts: (1) P-N-U-N-U-N-P and (2) U-N-P-N-P-N-U. The order of these two blocks was counterbalanced across the subjects. A total of 12 shocks were administered during each FPS test session.

The shocks were delivered through two medal electrodes located on the inner side of one of the subjects' forearms. Shock stimuli were delivered using a Digitimer DS7A constant current stimulator (Digitimer Ltd, Hertfordshire, England). Stimulation consists of short trains (total duration maximally 750 ms) of brief (2 ms) pulses. The maximum current intensity delivered during the study was 7 mA.

STATISTICAL ANALYSIS

PHARMACOKINETICS

The plasma concentrations of pregabalin, diphenhydramine and alprazolam were summarized by time points, and graphically presented as mean concentration-time profiles. The error bars represent the standard deviation (SD) at each time point.

PHARMACODYNAMICS

Body sway values were log-transformed prior to analysis to correct for the expected log-normal distribution of the data [17-19]. The effects of the four treatments on the pharmacodynamic measurements were compared with a mixed model analysis of variance. In this statistic model, treatment, period, time and treatment by time were set as fixed factors; and the random factors were subject, subject by treatment and subject by time; the baseline value was included as covariate, where baseline is defined as the average of the available measures obtained prior to dosing. The following contrasts were requested to demonstrate the effects of the active treatments: placebo-pregabalin, placebo-alprazolam, and placebo-diphenhydramine.

A summary table of the analysis results was generated with estimates of the difference between each active treatment and placebo and a back-transformed estimate of the difference in percentage for Body Sway, 95% confidence intervals (in percentage for Body Sway) and Least Square Means (geometric means for Body Sway), and the p-value of the contrasts. Least Square Means graphs were generated, with the Least Square Means of the analysis of the data as change from baseline.

Previous studies suggested good sensitivity of SPV to the effect of BZDs [6] and $\alpha_{2,3}$ subtype-selective GABA-A receptor modulators [17-19,33-35]. There is a close association between the effect size of benzodiazepines for SPV-reduction and their administered doses [6]. Based on the putative link between GABA-A $\alpha_{2,3}$ receptors and anxiety [36,37], this supports the consideration of SPV as a biomarker of clinical anxiolysis associated with GABA $\alpha_{2,3}$ activation [16], and the predictivity of SPV was supported by the selective SPV-reduction caused by TPA023 [17], combined with early clinical findings of this partial GABA $\alpha_{2,3}$ agonist [36]. BZDs also affected body sway, $VAS_{alertness}$, adaptive tracking, and $VAS_{calmness}$, suggesting impairment of postural balance, subjective alertness, eye-hand coordination, and subjective calmness, respectively [17-19,33,34]. Given the clinical relevance of these pharmacodynamic parameters, scatter plots of each pharmacodynamic measurement against simultaneously obtained SPV values were depicted to demonstrate SPV-normalized effect profiles with the study treatments. Moreover, a regression analysis was performed using the mixed model with treatment as the fixed factor

and SPV change from baseline and intercept as the random factors. Comparisons were made between each two active treatments with regards to the estimates of the slopes of the regression line obtained from each relative effect profile. The estimates of the slopes and their estimated difference were tabulated with the p-values. The slopes of these regression lines can be regarded as a measure of pharmacological selectivity of the drugs in respective of their anxiolytic effect [16].

RESULTS

SUBJECTS

Twelve men and ten women participated in the study. Ten subjects of each gender completed the study. The two drop-outs withdrew for personal reasons unrelated to the study, and were replaced by male subjects who received the same order of study treatments. Subjects had an average age of 22 years (range 18-36), and BMI of 23.3 kg/m^2 (range 18.1-29.6). Data from all treated subjects were used in the analyses of safety and pharmacokinetics. Subjects who completed the study per protocol were included in the pharmacodynamic analysis.

SAFETY

No serious adverse events were observed during the study. Neither were subjects discontinued their study due to AEs. The most frequently reported adverse events were 'somnolence', 'dizziness', 'fatigue' and 'headache'. Alprazolam was associated with the largest number of CNS-related AEs (n=21 in 14 out of 21 [66.7%] subjects), followed by diphenhydramine (n=19 in 16 out of 21 [76.2%] subjects), pregabalin (n=15 in 9 out of 20 [45.0%] subjects) and placebo (n=14 in 11 out of 20 [55.0%] subjects). Most AEs were attributed to the CNS-depressant effects of the study treatments. No ECG or laboratory abnormalities were judged clinically significant.

PHARMACOKINETICS

Sixty-two concentration-time profiles were obtained (20 for pregabalin, 21 for diphenhydramine and 21 for alprazolam). Following single-dose oral administration, peak plasma concentrations of all three active treatments were reached at 2-3 hours post-dose. Mean (standard deviation, SD) C_{max} was 4.87 (0.94), 91.47 (29.85) and 15.17 (2.10) mg/L for pregabalin, diphenhydramine and alprazolam, respectively. Figure 1 showed the average concentration-time profiles of pregabalin, diphenhydramine and alprazolam.

PHARMACODYNAMICS

The profiles of the CNS pharmacodynamic parameters (Figure 2 and Figure 3) showed that peak effects of the study treatments were usually observed around the point of T_{max}. Table 1 summarized the results of statistical comparisons between each active drug and placebo. Compared to placebo, $VAS_{calmness}$ increased statistically significantly with alprazolam (2.0 mm) and pregabalin (2.5 mm), but not with diphenhydramine (1.1 mm). In the meantime, saccadic peak velocity (SPV) declined after alprazolam (-57 deg/sec) and pregabalin (-28 deg/sec), more than by diphenhydramine (-14 deg/sec); so did smooth pursuit. The average responses of SPV were significantly correlated with the drug-induced increases in $VAS_{calmness}$.

To further characterize the pharmacodynamic profiles of these compounds, various CNS pharmacodynamic effects were compared with the corresponding drug-induced SPV reductions. According to the analyses about SPV-relative effect profiles (Table 2), the SPV-normalized impairment of adaptive tracking was higher after diphenhydramine and alprazolam, compared to that of pregabalin. The estimated slope for the regression line $\Delta sway/\Delta SPV$ was rather flat with pregabalin and significantly smaller than alprazolam and diphenhydramine. The slope for the $\Delta VAS_{alertness}/\Delta SPV$ relation was larger with pregabalin and alprazolam than with diphenhydramine. No significant difference was found among alprazolam, diphenhydramine, and pregabalin in the relative effect profiles of $\Delta VAS_{calmness}$ versus ΔSPV. The results of the FPS paradigm were reported in a separate article [22].

DISCUSSION

In this study, a set of neuropsycho-pharmacodynamic tests (i.e., the Neurocart battery) was performed to characterize the CNS profiles of three clinically anxiolytic and/or hypnotic drugs. Therapeutically relevant doses were administered as a single dose, because all drugs had a rapid onset of effects. The aim was to identify response patterns that are shared by fast-acting anxiolytics (alprazolam and pregabalin) but differ from sedative effects (diphenhydramine).

For the assessment of fear-potentiated startle, none of the treatments reliably reduced either fear- or anxiety-potentiated startle. Alprazolam and diphenhydramine reduced overall baseline startle. Pregabalin did not significantly affect any of the physiological measures [22]. On the other hand, as a full GABA-A agonist, alprazolam induced robust effects on most CNS parameters. Such generalized CNS depressive pharmacodynamics is similar to that of other benzodiazepines [29,33,34] and can be explained by the non-selective modulation of alprazolam on

different GABA-A receptor subtypes, which constitute the most widely distributed inhibitory receptors in the CNS. Pregabalin and its congener gabapentin are more selective and affect the α2 subunit of the voltage-dependent calcium channel. Contrary to benzodiazepines, 'gabapentinoids' don't bind to GABA receptors, but both drug classes lead to a decrease of the stimulatory neurotransmitters that are involved in anxiety, such as glutamate and the monoamines [38]. In this study, pregabalin was associated with moderate reduction of SPV and smooth pursuit, as well as statistically significant increase of $VAS_{calmness}$. Diphenhydramine, acting as an antagonist at the histamine H_1 receptors, slightly reduced SPV, but it did not influence $VAS_{calmness}$. As an indication that the 50 mg dose was functionally relevant, diphenhydramine showed a prominent effect on adaptive tracking.

An important finding of this study was the improvement of subjective calmness after a single dose of pregabalin and alprazolam. Moreover, the increase of $VAS_{calmness}$ was significantly correlated with SPV reductions. The literature is less clear about the subjective effects of anxiolytic drugs in healthy volunteers. In general, inconsistent changes of $VAS_{calmness}$ have been reported for single doses of lorazepam (2 mg) and some $\alpha_{2,3}$-subtype selective GABA-A agonists [17-19,33,34], even at dosages that are clinically more anxiolytic than the relatively low doses of alprazolam 1 mg or pregabalin 200 mg employed in the current study. These inconsistencies suggest that $VAS_{calmness}$ is a less reliable biomarker in studies where anxiety is not specifically stimulated. In such 'normal' drug studies, healthy subjects can experience different levels of anxiety, for instance depending on how familiar they are with these experiments, which may affect their sensitivity to anxiolytic drug effects. In the current study, subjects were repeatedly exposed to fear potentiated startle tests, which include unpleasant electrical shocks. We assume that this has induced a mild anticipatory anxiety in the study subjects [39], which was suppressed by the anxiolytic drugs but not by the sedative antihistamine.

On the other hand, the partial effect profiles of diphenhydramine and pregabalin and the more general CNS depression caused by alprazolam seems to match their pharmacological characteristics. Strictly speaking, a reliable comparison of pharmacological effect profiles is only justified across a wider dose range or at least at roughly equipotent dosages. Although it is difficult to establish dose equivalence across different drugs classes, all doses were in their therapeutic range. We tried to solve this further by looking at relative effect profiles across the entire profile of the plasma concentrations of the investigated drugs [16]. With this approach, the concern regarding dose equivalence in PD comparisons is overcome by transforming from dose-based PD-effect relationship to exposure-based PD effect relationship. SPV is one of the most sensitive pharmacodynamic biomarkers for anxiolytic doses of benzodiazepines [6]. Therefore, SPV was used to benchmark anxiolytic effects and was compared by linear regression with a second CNS biomarker to depict a drug effect on another CNS domain.

As can be seen in Table 1 and 2, alprazolam and diphenhydramine lead to comparable impairments on body sway (measure of postural stability) relative to their effects on saccadic peak velocity. In contrast, the effect of pregabalin on body sway was less remarkable than SPV. The differential effects of pregabalin on these two pharmacodynamic parameters seem to be consistent with the clinical behavior of this compound, which, compared to benzodiazepines, shows a larger therapeutic window between anxiolysis and ataxia [40]. The slopes of the $\Delta VAS_{alertness}/\Delta SPV$ regression lines are comparable among the study treatments. This is different from our previous findings between selective and non-selective GABA-A receptor agonists [16]. As subjects were physically and mentally stressed by electronic shocks of the fear-potentiated-startle paradigm [22], this challenge probably increased the baseline level of $VAS_{alertness}$ and hence reduced the responses to the investigated anxiolytic/hypnotic drugs. In addition, a distinct relationship was seen in the ΔSPV-relative effect profiles of ΔTracking among the three compounds. The steeper slope of the ΔTracking/ΔSPV regression line after diphenhydramine reflects its minimal effect on SPV but substantial effect on tracking. Such a profile is linked to the clinical properties of diphenhydramine: it shows considerable hypnotic effects at the dose of 50 mg, but does not lead to anxiety relief. Known side-effects of this compound, including drowsiness and motor impairment, are attributed to its inverse agonism at the histamine H_1-receptors distributed in the brain.

Taken together, the results of present study supports the combination a physically stressful procedure to the subjective assessment of anxiolysis. Consistently, the simultaneous reduction of SPV and the correlation between these two PD measurements provide further confirmation for the use of these biomarkers for clinically relevant anxiolytic effects. The sensitivity of the experiment appears to have been increased by the constant mild anticipation of shock during repeated FPS testing. The different effect profiles of the three drugs are in line with their pharmacological distinctions. These findings corroborate the profiling of CNS effects to demonstrate pharmacological selectivity, optimize the previous use of EEG/psychomotor/subjective pharmacological assessments [41] to a more pharmacological mechanism-based PD marker selection, and warrant the extension from a single, less reliable, subjective assessment to the combination of a stress-challenged subjective measurement and a neurophysiological test for the evaluation and extrapolation of clinical anxiolysis.

REFERENCES

1 Greenblatt DJ, Wright CE. Clinical pharmacokinetics of alprazolam. Therapeutic implications. Clin Pharmacokinet. 1993 Jun; 24(6): 453-71.

2 Cohen AF, Burggraaf J, Van Gerven JMA, Moerland M, Groeneveld GJ. The Use of Biomarkers in Human Pharmacology (Phase I) Studies. Annu Rev Pharmacol Toxicol 2015; 6; 55: 55-74

3 Cohen AF. Developing drug prototypes: pharmacology replaces safety and tolerability? Nat Rev Drug Discov. 2010; 9: 856-65.

4 Morgan P, Van Der Graaf PH, Arrowsmith J, Feltner DE, Drummond KS, Wegner CD, Street SD. Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. Drug Discov Today. 2012; 17(9-10): 419-24
5 Dumont GJH, De Visser SJ, Cohen AF, Van Gerven JMA. Biomarkers for the effects of selective serotonin reuptake inhibitors (SSRIs) in healthy volunteers. Br J Clin Pharmacol 2005; 59: 495-510 (Review).
6 De Visser SJ, Van der Post JP, De Waal PP, Cornet F, Cohen AF, Van Gerven JMA. Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 2003;55:39-50 (Review)
7 McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR, Farrar S, Myers J, Cook G, Ferris P, Garrett L, Bristow L, Marshall G, Macaulay A, Brown N, Howell O, Moore KW, Carling RW, Street LJ, Castro JL, Ragan CI, Dawson GR, Whiting PJ. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A)receptor alpha1 subtype. Nat Neurosci. 2000 Jun;3(6):587-92.
8 Rudolph U, Crestani F, Benke D, Brünig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H, Möhler H. Benzodiazepine actions mediated by specific gamma-aminobutyric acid (A) receptor subtypes. Nature. 1999 Oct 21;401(6755): 796-800. Erratum in: Nature 2000 Apr 6;404(6778):629.
9 Crestani F, Keist R, Fritschy JM, Benke D, Vogt K, Prut L, Blüthmann H, Möhler H, Rudolph U. Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors. Proc Natl Acad Sci U S A. 2002 Jun 25; 99(13): 8980-5.
10 Laurijssens BE, Greenblatt DJ. Pharmacokinetic-pharmacodynamic relationships for benzodiazepines. Clin Pharmacokinet. 1996 Jan; 30(1): 52-76.
11 Besson M, Matthey A, Daali Y, Poncet A, Vuilleumier P, Curatolo M, Zeilhofer HU, Desmeules J. GABAergic modulation in central sensitization in humans: a randomized placebo-controlled pharmacokinetic-pharmacodynamic study comparing clobazam with clonazepam in healthy volunteers. Pain. 2015 Mar;156(3):397-404.
12 Tsunoda K, Uchida H, Suzuki T, Watanabe K, Yamashima T, Kashima H. Effects of discontinuing benzodiazepine-derivative hypnotics on postural sway and cognitive functions in the elderly. Int J Geriatr Psychiatry. 2010 Dec; 25(12): 1259-65.
13 Reilly JL, Lencer R, Bishop JR, Keedy S, Sweeney JA. Pharmacological treatment effects on eye movement control. Brain Cogn. 2008 Dec;68(3):415-35.
14 Mandema JW, Danhof M. Electroencephalogram effect measures and relationships between pharmacokinetics and pharmacodynamics of centrally acting drugs. Clin Pharmacokinet. 1992 Sep; 23(3): 191-215.
15 van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. J Psychopharmacol. 1999;13: 10-17.
16 Chen X, de Haas S, de Kam M, van Gerven J. An overview of the CNS-pharmacodynamic profiles of non-selective and selective GABA receptor modulators. Adv Pharmacol Sci. 2012;2012:134523. doi: 10.1155/2012/134523.
17 de Haas SL, de Visser SJ, van der Post JP, de Smet M, Schoemaker RC, Rijnbeek B, Cohen AF, Vega JM, Agrawal NG, Goel TV, Simpson RC, Pearson LK, Li S, Hesney M, Murphy MG, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of TPA023, a GABA-A a2,3 subtype-selective receptor modulator, compared to lorazepam and placebo in healthy volunteers. Journal of Psychopharmacology. 2007, 21; 374-383.
18 de Haas SL, de Visser SJ, van der Post JP, Schoemaker RC, van Dyck K, Murphy MG, de Smet M, Vessey LK, Ramakrishnan R, Xue L, Cohen AF, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of MK-0343, a GABA-A $\alpha_{2,3}$ subtype selective receptor modulator, compared to lorazepam and placebo in healthy male volunteers. J Psychopharmacology. 2008; 22: 24-32.
19 de Haas SL, Franson KL, Schmitt JA, Cohen AF, Fau JB, Dubruc C, van Gerven JM. The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA-A 2,3 selective receptor modulator, in comparison with lorazepam in healthy volunteers. J Psychopharmacol. 2009;23: 625-632.
20 Buoli M, Dell'osso B, Bosi MF, Altamura C. Slow vs standard up-titration of paroxetine in the treatment of panic disorder: a prospective randomized trial. Psychiatry Clin Neurosci. 2010;64(6): 612-9.
21 Blin O, Micallef J, Audebert C, Legangneux E. A double-blind, placebo- and flurazepam-controlled investigation of the residual psychomotor and cognitive effects of modified release zolpidem in young healthy volunteers. J Clin Psychopharmacol. 2006 Jun;26(3): 284-9.
22 Baas JPM, Mol N, Kenemans JL, Prinssen EP, Niklson I, Chen X, Broeyer F, Van Gerven J. Validating a human model for anxiety using startle potentiated by cue and context: the effects of alprazolam, pregabalin, and diphenhydramine. Psychopharmacology (Berl) 2009;205: 73-84.
23 Grillon C, Baas JM, Pine DS, Lissek S, Lawley M, Ellis V, Levine J. The benzodiazepine alprazolam dissociates contextual fear from cued fear in humans as assessed by fear-potentiated startle. Biol Psychiatry. 2006; 60(7): 760-6.
24 Hermans EJ, Putman P, Baas JM, Koppeschaar HP, van Honk J. A single administration of testosterone

reduces fear-potentiated startle in humans. Biol Psychiatry. 2006; 59(9): 872-4.
25 Baas JM, Grillon C, Böcker KB, Brack AA, Morgan CA 3rd, Kenemans JL, Verbaten MN. Benzodiazepines have no effect on fear-potentiated startle in humans. Psychopharmacology (Berl). 2002 May; 161(3): 233-47.
26 Wright BM. A simple mechanical ataxia-meter. J Physiol. 1971;218 Suppl:27P-28P.
27 van Steveninck AL, Gieschke R, Schoemaker RC, Roncari G, Tuk B, Pieters MS, Breimer DD, Cohen AF. Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. Br J Clin Pharmacol. 1996 ;41: 565-573.
28 Norris H. The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology. 1971; 10: 181-91.
29 van Steveninck AL, Schoemaker HC, den Hartigh J, Pieters MS, Breimer DD, Cohen AF. Effects of intravenous temazepam. II. A study of the long-term reproducibility of pharmacokinetics, pharmacodynamics, and concentration-effect parameters. Clin Pharmacol Ther. 1994; 55: 546-55.
30 Bittencourt PR, Wade P, Smith AT, Richens A. The relationship between peak velocity of saccadic eye movements and serum benzodiazepine concentration. Br J Clin Pharmacol. 1981;12: 523-533.
31 Baloh RW, Sills AW, Kumley WE, Honrubia V. Quantitative measurement of saccade amplitude, duration, and velocity. Neurology. 1975; 25: 1065-1070.
32 Borland RG and Nicholson AN. Visual motor co-ordination and dynamic visual acuity. Br J Clin Pharmacol. 1984;18 Suppl:69S-72S.
33 Zuiker RG, Chen X, Østerberg O, Mirza NR, Muglia P, de Kam M, Klaassen ES, van Gerven JM. J Psychopharmacol. NS11821, a partial subtype-selective GABA-A agonist, elicits selective effects on the central nervous system in randomized controlled trial with healthy subjects. 2016 Mar;30(3): 253-62.
34 Chen X, Jacobs G, de Kam ML, Jaeger J, Lappalainen J, Maruff P, Smith MA, Cross AJ, Cohen A, van Gerven J. AZD6280, a novel partial γ-aminobutyric acid A receptor modulator, demonstrates a pharmacodynamically selective effect profile in healthy male volunteers.J Clin Psychopharmacol. 2015 Feb;35(1): 22-33.
35 Chen X, Jacobs G, de Kam M, Jaeger J, Lappalainen J, Maruff P, Smith MA, Cross AJ, Cohen A, van Gerven J. The central nervous system effects of the partial GABA-A$\alpha_{2,3}$-selective receptor modulator AZD7325 in comparison with lorazepam in healthy males. Br J Clin Pharmacol. 2014 Dec;78(6): 1298-314.
36 Atack JR. GABA-A receptor alpha2/alpha3 subtype-selective modulators as potential nonsedating anxiolytics. Curr Top Behav Neurosci. 2010;2: 331-60
37 Engin E, Liu J, Rudolph U. α2-containing GABA(A) receptors: a target for the development of novel treatment strategies for CNS disorders. Pharmacol Ther. 2012 Nov;136(2): 142-52
38 Micó JA, Prieto R. Elucidating the mechanism of action of pregabalin: α(2)Δ as a therapeutic target in anxiety. CNS Drugs 2012;26: 637-48.
39 Robinson OJ, Vytal K, Cornwell BR, Grillon C. The impact of anxiety upon cognition: perspectives from human threat of shock studies. Front Hum Neurosci. 2013 May 17;7: 203.
40 Gilron I. gabapentin and pregabalin for chronic neuropathic and early postsurgical pain: current evidence and future directions. Curr Opin Anaesthesiol. 2007 Oct;20(5): 456-72.
41 Laurijssens BE, Greenblatt DJ. Pharmacokinetic-pharmacodynamic relationships for benzodiazepines. Clin Pharmacokinet. 1996 Jan; 30(1): 52-76.

Table 1 · Summary of the analysis results for CNS-pharmacodynamic parameters. (The results are presented as the estimated differences between each active treatment and placebo in the least square mean [LSM] change from baseline and the 95% confidence intervals [CIs] of the differences. The results of body sway are presented as the differences of LSM proportional change from baseline and their 95% CIs.)

Parameter (unit)	Pregabalin vs. Placebo	Alprazolam vs. Placebo	Diphenhydramine vs. Placebo
Body sway (mm)	12.27% (-2.37%, 29.11%) p=0.1026	34.43% (16.90%, 54.59%) p<0.0001	12.25% (-2.35%, 29.03%) p=0.1021
Saccadic Inaccuracy (%)	0.4 (-0.2, 0.9) p=0.1670	0.8 (0.3, 1.4) p=0.0021	0.3 (-0.2, 0.8) p=0.1827
Saccadic Peak Velocity (deg/sec)	-27.7 (-35.9, -19.5) p<0.0001	-56.9 (-65.0, -48.8) p<0.0001	-13.8 (-21.7, -5.9) p=0.0010
Saccadic Reaction Time (sec)	0.001 (-0.006, 0.009) p=0.7032	0.010 (0.003, 0.017) p=0.0082	0.002 (-.005, 0.009) p=0.6109
Smooth pursuit (%)	-5.1 (-7.8, -2.5) p=0.0003	-6.8 (-9.5, -4.2) p<0.0001	-0.5 (-3.1, 2.1) p=0.7149
Adaptive tracking (%)	-1.04 (-2.30, 0.22) p=0.1039	-5.04 (-6.30, -3.78) p<0.0001	-2.64 (-3.92, -1.36) p=0.0001
VAS Alertness (mm)	-2.3 (-5.7, 1.0) p=0.1676	-4.5 (-7.8, -1.1) p=0.0096	-1.0 (-4.4, 2.3) p=0.5377
VAS Calmness (mm)	2.5 (0.4, 4.7) p=0.0201	2.0 (-0.1, 4.1) p=0.0606	1.1 (-1.0, 3.2) p=0.3066
VAS Mood (mm)	0.7 (-0.5, 2.0) p=0.2483	-0.1 (-1.4, 1.1) p=0.8633	0.4 (-0.8, 1.7) p=0.5059

CNS=central nervous system

Table 2 · Summary of Relative Effect Profile Among The Three Active Treatments. (The results are presented as least square mean [LSM] estimates of the slope of regression line. The p-values are presented for the comparisons of each two active treatments.)

				P-value		
	ALP	DPH	PRG	ALP-DPH	ALP-PRG	DPH-PRG
ΔSway/ΔSPV	-0.00208	-0.00186	-0.00106	0.5733	0.0055	0.0716
ΔTracking/ΔSPV	0.07785	0.06189	0.03056	0.1526	<0.0001	0.0133
ΔVAS alertness/ΔSPV	0.07227	0.01491	0.06061	0.0008	0.4540	0.0156
ΔVAS calmness/ΔSPV	-0.03626	-0.02776	-0.05070	0.6564	0.4123	0.2834

Figure 1 · Average plasma concentration-time profiles with standard deviation (SD) error bars of each compound after single oral administration (population estimates superimposed)

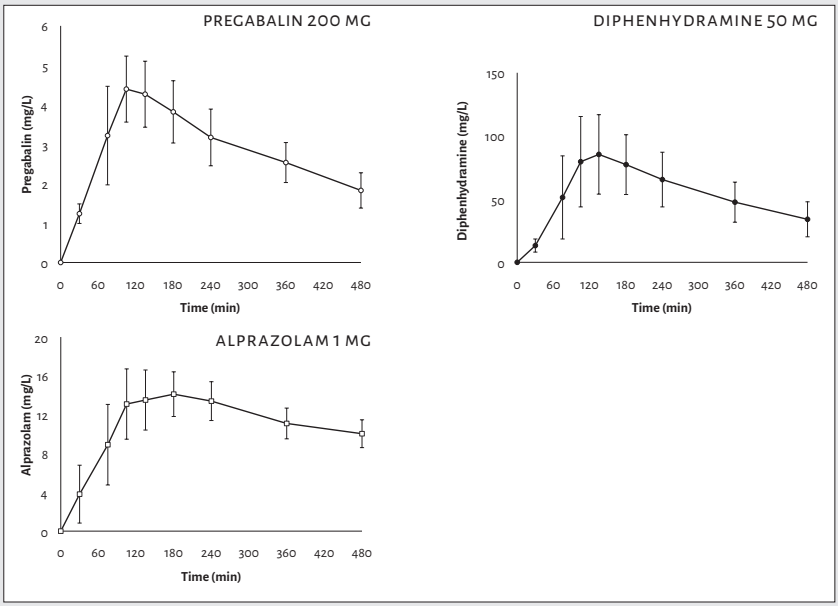
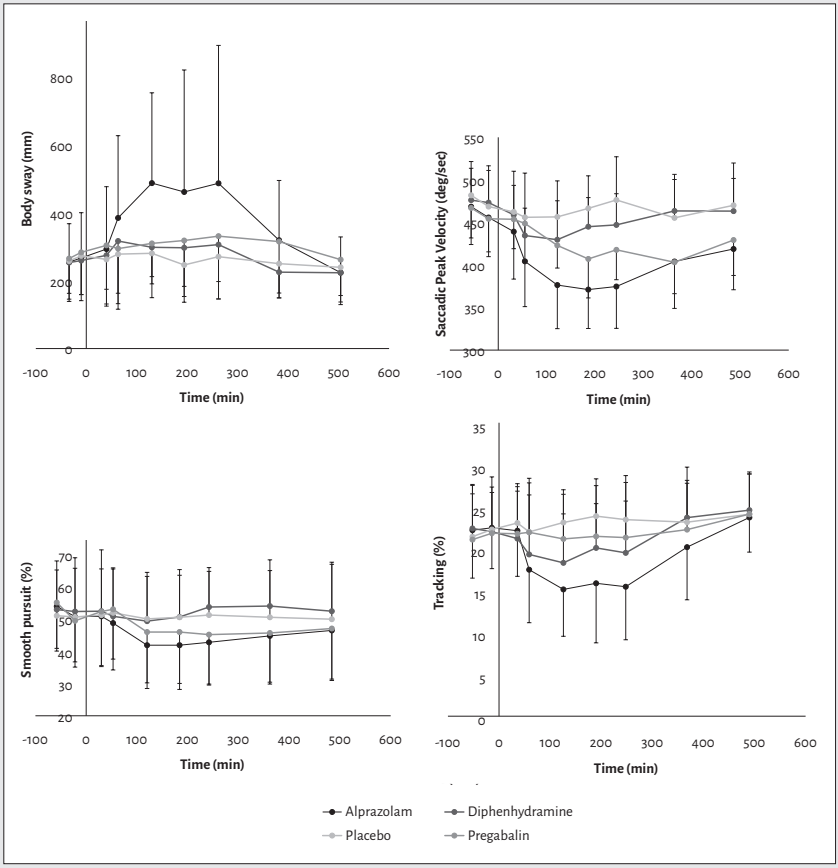
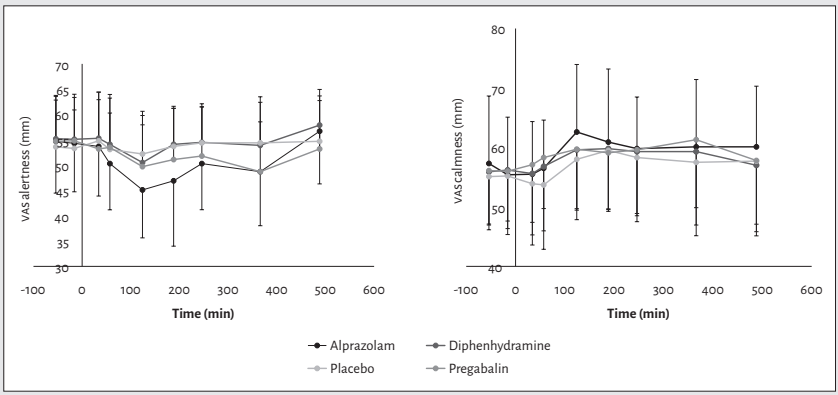


Figure 2 · Graph of means of objective CNS-pharmacodynamic parameters with standard deviation as error bars



2a. Body Sway; 2b. Saccadic Peak Velocity; 2c. Smooth Pursuit; 2d. Adaptive Tracking

Figure 3 · Graph of means of subjective CNS-pharmacodynamic parameters with standard deviation as error bars



3a. Visual Analogue Scale of Alertness; 3b. Visual Analogue Scale of Calmness