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

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VI

THE EFFECTS OF THE NONSELECTIVE BENZODIAZEPINE LORAZEPAM AND THE α2/α3 SUBUNIT-SELECTIVE GABA-A RECEPTOR MODULATORS AZD7325 AND AZD6280 ON PLASMA PROLACTIN LEVELS

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ABSTRACT

Compounds with selectivity for GABA-A receptor subtypes may differ significantly from nonselective benzodiazepines in their dopaminergic effects in vivo. To explore the exact role of the GABA-A receptor subtypes in the regulation of prolactin secretion and the differential effects of selective and nonselective GABA receptor modulators, the effects of the nonselective benzodiazepine lorazepam, as well as two novel α_2/α_3 subunit-selective GABA-A receptor modulators AZD7325 and AZD6280, on prolactin levels were measured in healthy male volunteers. Following administration of lorazepam at 2 mg doses and AZD6280 at 10 mg and 40 mg doses, prolactin levels increased significantly compared with placebo (difference 42.0%, 19.8% and 32.8% respectively), suggesting that the α_2 and/or α_3 receptor subtypes are involved in GABAergic modulation of prolactin secretion, although possible roles of the α_1 and α_5 receptor subtypes are not excluded. The increases in prolactin levels after administration of AZD7325 at 2 mg and 10 mg doses (difference 7.6% and 10.5% respectively) did not reach statistical significance, suggesting that doses of AZD7325 or intrinsic efficacy at the α_2 and α_3 receptor subtypes may have been too low.

INTRODUCTION

A series of studies in animal models has indicated that certain effects of benzodiazepines may be attributable to efficacy at specific GABA-A receptor subtypes, such as sedation (α_1 receptor subtype) [1, 2], anxiolysis (α_2 and α_3 receptor subtypes) [3-6] and learning and memory (α_5 receptor subtype) [7, 8]. Accordingly, it has been suggested that compounds with high efficacy at α_2 and/or α_3 receptor subtypes and low efficacy at α_1 and α_5 receptor subypes may exhibit significant anxiolysis, with less sedation than nonselective benzodiazepines [9]. In addition, because γ-aminobutyric acid (GABA) is one of the major inhibitors of dopamine neurotransmission, it has also been suggested that compounds with selectivity for GABA-A receptor subtypes may differ significantly from nonselective benzodiazepines in their effects on dopamine neurotransmission [9]. In the ventral tegmental area, dopaminergic neurons express α_3 receptor subtypes, whereas GABAergic interneurons express α_1 receptor subtypes [10]. Benzodiazepines inhibit the dopaminergic neurons through efficacy at α_3 receptor subtypes, but simultaneous efficacy at α_1 receptor subtypes on the GABAergic interneurons results in disinhibition of the dopaminergic neurons [9, 10]. Thus, compounds with high efficacy at α_3 receptor subtypes and low efficacy at α_1 receptor subtypes may attenuate dopamine neurotransmission without counteractive disinhibition. Such compounds may have therapautic potential in disorders such as schizophrenia [9].

To evaluate if compounds with selectivity for GABA-A receptor subtypes differ from nonselective benzodiazepines in their dopaminergic effects in vivo, we evaluated the effects of lorazepam and two novel selective modulators of α_2 and α_3 receptor subtypes on the activity of the tuberoinfundibular pathway, which represents the most readily available dopaminergic pathway for evaluation in vivo, by measuring circulating prolactin levels. The tuberoinfundibular dopaminergic pathway is the primary physiological inhibitory control mechanism of prolactin secretion [11, 12]. GABA may directly inhibit the activity of the hypothalamic tuberoinfundibular dopaminergic pathway, with a resulting increase in prolactin secretion [12-16]. It has also been reported that GABA exerts a dual control [17] and can also have an inhibitory effect on prolactin release by acting at GABA receptors in the anterior pituitary gland [12, 16], but this effect is less clear *in vivo* [18] than *in vitro*. Only a few studies have evaluated the direct effects of GABAergic drugs on circulating basal prolactin levels in healthy subjects. Diazepam [19, 20] and bromazepam [21] did not significantly affect prolactin levels, while temazepam was found to increase prolactin levels only to a small extent [22]. Alprazolam at high doses increased prolactin levels [23], while lower doses had no significant effect [24]. Zolpidem and bretazenil stimulated nocturnal secretion of prolactin [25, 26], while sodium valproate decreased prolactin levels [27]. The effect sizes in these studies, if any, were very small, especially compared with the potent prolactin-elevating effects of dopamine D_2 receptor antagonists.

To explore the exact role of the various GABA-A receptor subtypes in the regulation of prolactin secretion and the differential effects of selective and nonselective GABA receptor modulators, we report the effects of two novel α_2/α_3 subunit-selective GABA-A receptor modulators, AZD7325 [28, 29] and AZD6280 [28, 30], and a therapeutic dose of lorazepam on prolactin secretion. These studies were part of larger phase I pharmacokinetic and pharmacodynamic studies of these compounds, which will be reported elsewhere [31, 32].

METHODS

STUDY DESIGN

In total, 32 healthy male volunteers were planned to participate in two parallel double-blind, placebo-controlled, randomized, cross-over studies. To be eligible for inclusion in both studies, subjects were required to be aged between 18 and 55 years, with a body mass index (BMI) of 18 to 30 kg/m² and refrain from alcoholic beverages, smoking and caffeine-containing products during study days. Both studies were approved by the medical ethics review board of the Leiden University Medical Center. Prior to medical screening, all subjects gave written informed consent. Both studies had an identical design, except the administered drugs. In the first study, 16 subjects were administered single oral doses of 2 mg lorazepam, 2 mg AZD7325, 10 mg AZD7325 or placebo, during four separate study periods. In the second study, 16 subjects were administered single oral doses of 2 mg lorazepam, 10 mg AZD6280, 40 mg AZD6280 or placebo, during four separate study periods. Study periods were scheduled in randomized order using a Williams Latin square design and were separated by a washout time of at least 7 days. On study days, subjects fasted for minimally 2.5 hours after a light standard breakfast until dose administration (which generally occured between 11h00m and 12h00m AM) and continued fasting until 4 hours after dose administration.

POWER CALCULATION

A power calculation using data from a previous study [33] indicated that the present study (n = 32 subjects receiving lorazepam, power 80% and alpha 0.05) was powered to detect an increase of 12.5% or a decrease of 11% in prolactin concentration after administration of lorazepam, compared with placebo.

PLASMA CONCENTRATION OF PROLACTIN

Venous blood samples for analysis of prolactin concentration were collected prior to study drug administration and at ½, 1, 1¼, 1½, 2, 2½, 3¼, 4, 4½, 6, 8, 12 and 21 hours

after study drug administration. Plasma concentrations of prolactin were determined using an electrochemiluminescence immunoassay (ECLIA) with a lower detection limit of 0.047 ng/mL (Elecsys Prolactin II assay, Roche Diagnostics GmbH, Mannheim, Germany).

STATISTICAL ANALYSIS

Prolactin measurements up to 8 hours after administration of lorazepam or placebo were compared with a mixed model analysis of variance with treatment, period, time and treatment by time as fixed factors, and subject, subject by treatment and subject by time as random factors, and the pre-value (measurement prior to study drug administration) as covariate. Prolactin measurements were log-transformed prior to analysis to correct for the log-normal distribution of the data. Estimates of treatment differences and back-transformed estimates of the difference in percentage with corresponding 95% confidence intervals (95% CI) and *p*-values were calculated. All calculations were performed using SAS for Windows version 9.1.3 (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

SUBJECTS

Subjects had a mean age of 28.1 years (range 18-54), weight of 76.1 kg (range 62.0-89.5) and body mass index (BMI) of 23.0 kg/m^2 (range 19.1-26.7). Two subjects withdrew informed consent after completion of study period 1 for reasons unrelated to study drug administration. Another subject tested positive for THC in study period 2 and was excluded from participation. Pharmacodynamic data from these subjects were not used for further analysis. All three subjects were replaced. Therefore, in total, 32 subjects completed the study.

PLASMA CONCENTRATION OF PROLACTIN

Plasma concentrations of prolactin after administration of lorazepam, AZD7325 and AZD6280 are shown in Figure 1 and Table 1. Following administration of 2 mg lorazepam, prolactin levels increased with 42.0% compared with placebo (95% CI 31.4/53.5%, $p < 0.001$), which remained elevated until at least 8 hours after dose administration. Following administration of 2 mg and 10 mg AZD7325, prolactin levels increased with 7.6% and 10.5%, respectively, compared with placebo. Both increases did not reach statistical significance, although the 10.5% increase after the 10 mg dose has a *p*-value of 0.0536. Following administration of 10 mg AZD6280, prolactin levels increased significantly compared with placebo (difference 19.8%

versus placebo, 95% CI 8.2/32.6%, $p = 0.0007$). A larger increase was observed after administration of 40 mg of AZD6280 (difference 32.8% versus placebo, 95% CI 20.0/47.0%, $p < 0.0001$). Prolactin levels after administration of lorazepam were significantly higher than those after AZD7325 at 2 and 10 mg doses and AZD6280 at 10 mg doses, but were not significantly different from those after AZD6280 at 40 mg doses.

DISCUSSION

Compounds with high efficacy at α_2 subunit-containing GABA-A receptor subtypes and low efficacy at α_1 receptor subtypes may differ significantly from nonselective benzodiazepines in their effects on dopaminergic circuits [9]. Such compounds may thus have therapeutic potential in disorders such as (certain aspects of) schizophrenia [9]. The present study was performed to evaluate the effects of two novel α_2/α_3 subunit selective GABAergic drugs on the activity of the tuberoinfundibular dopaminergic pathway, by measuring circulating prolactin levels in healthy male volunteers, compared with lorazepam and placebo.

After administration of placebo, prolactin levels showed an initial decrease with a return to baseline values at the end of the study day, which is consistent with a normal circadian rhythm [12, 34]. Also, a peak in prolactin levels was observed 6 hours after dose administration, which probably reflects normal prolactin release following food consumption [35, 36.]

After administration of a single oral dose of 2 mg lorazepam, an increase of 42.0% in prolactin levels was observed. The magnitude of the effects of lorazepam on prolactin levels was rather small, especially in comparison to the much more potent prolactin-elevating effects of dopamine D_2 receptor antagonists. Haloperidol at 3 mg doses increases prolactin levels with 130.9% [37]. Thus, the effects of lorazepam administration on prolactin secretion are not likely to produce clinically relevant hyperprolactinaemia in men. However, our studies showed clear results in comparison with other studies that evaluated the effects of GABAergic drugs on basal prolactin levels in healthy subjects. The benzodiazepines diazepam and bromazepam showed no significant effects on prolactin levels under resting conditions [19-21], whereas temazepam caused a small increase in prolactin levels of roughly 21.4 mU/L (which would correspond to roughly 1 ng/mL), but only at a single time point 1 hour after dose administration [22]. In contrast, our study demonstrates that lorazepam increases prolactin levels with roughly 5-6 ng/ml, which remain elevated until at least 8 hours after dose administration. The dose of lorazepam (2 mg) used in our study is roughly equipotent with the doses of diazepam (10 mg), bromazepam (3 mg) and temazepam (20 mg) used in these earlies studies, although estimates of benzodiazepine dose equivalencies differ somewhat

between various authors [38-40]. Dose dependency of the effects on prolactin secretion has been demonstrated with the benzodiazepine alprazolam, which causes an increase of prolactin levels with roughly 9-10 ng/mL at relatively high doses (3 mg) [23], while doses in the lower therapeutic range (0.5 mg) demonstrated no effects [24]. The different findings might be explained by the small sample sizes used in the earlier studies (6-10 subjects in most studies) and statistical power may thus have been too small.

The increase in prolactin levels following administration of the GABA agonist lorazepam in our study suggests that the postulated stimulatory effect of GABA transmission (by suppressing the tuberoinfundibular dopaminergic neurons in the hypothalamus) exceeds the postulated inhibitory effect of GABA transmission (directly at the anterior pituitary gland). The preferential effect of lorazepam on the tuberoinfundibular dopaminergic neurons might result from differences in affinity for the pituitary and hypothalamic GABA binding sites, as has been shown for the GABA agonist muscimol and antagonist bicuculline [41], both of which have higher affinity for the binding sites in the mediobasal hypothalamus than for the binding sites in the anterior pituitary. However, effects of benzodiazepines on the activity of the tuberoinfundibular dopaminergic neurons have not been confirmed *in vivo* in man. A recent positron emission tomography (PET) study using the dopamine D_2 receptor ligand [^{11}C]FLB457 in healthy subjects has demonstrated that single oral doses of 2.5 mg lorazepam induce a statistically significant decrease in dopamine D_2 receptor binding potential (BP_{ND}) in the medial temporal and dorsolateral prefrontal cortex [42], but effects on the hypothalamus were not reported. Although a decrease in BP_{ND} (i.e. suggesting dopamine release) in the cerebral cortex does not imply that lorazepam inhibits the tuberoinfundibular dopaminergic pathway in the hypothalamus, it does confirm that lorazepam can alter dopamine levels in extrastriatal areas in humans *in vivo*.

The present study evaluated the effects of two novel α_2/α_3 subunit-selective GABA-A receptor modulators, AZD7325 and AZD6280, on prolactin levels. Administration of 2 mg and 10 mg AZD7325 produced small increases in prolactin levels, which did not reach statistical significance, although the 10.5% increase after the 10 mg dose is in line with the other effects and has a *p*-value of 0.0536. Administration of 10 mg and 40 mg AZD6280 produced statistically significant increases in prolactin levels of 19.8% and 32.8%, respectively. These findings suggest that the α_2 and/or α_3 receptor subtypes are involved in GABAergic modulation of the tuberoinfundibular dopaminergic pathway. Indeed, α_2 and α_3 subunit-containing GABA-A recepors have been shown to be expressed in the arcuate nucleus and hypothalamus 43. However, it is not excluded that α_1 or α_5 receptor subtypes, which are also expressed in the arcuate nucleus and hypothalamus [43], are also involved in the control of prolactin secretion. The nonbenzodiazepine GABA agonist zolpidem (10 mg), which has modest selectivity for α_1 receptor subtypes [44],

increased nocturnal prolactin levels by two-fold 26. The effects of AZD7325 on prolactin secretion were less clear than those of AZD6280. Similarly, in other studies [31, 32], AZD7325 also caused fewer effects than AZD6280 on peak velocity of saccadic eye movements, which is one of the most consistent and sensitive biomarkers for the effects of nonselective benzodiazepines [45] and α_2/α_3 subtype-selective compounds [46] in healthy volunteers. These differences may be related to the lower dosages of AZD7325 used.

Comparison of the effects of 2 mg lorazepam with 40 mg AZD6280 indicated no statistically significant difference. The lower prolactin levels after AZD7325 at 2 and 10 mg doses and AZD6280 at 10 mg doses are likely related to dose. However, dose equivalencies of lorazepam, AZD7325 and AZD6280 are not known. Thus, these results do not fully exclude potential differences between nonselective benzodiazepines and selective α_2/α_3 subunit-containing GABA-A receptor modulators on prolactin secretion. In addition, our study measured prolactin levels only in healthy male volunteers. These results cannot readily be extrapolated to females, because the regulation of prolactin secretion in females is different, with the notable influence of estrogens.

In conclusion, the nonselective benzodiazepine lorazepam and the novel α_2/α_3 subunit-selective GABA-A receptor modulator AZD6280 at 40 mg doses both increase plasma prolactin levels in healthy male subjects. The increases in prolactin levels after administration of the novel α_2/α_3 subunit-selective GABA-A receptor modulator AZD7325 did not reach statistical significance, which may be related to the lower dosages used. These results indicate that the α_2 and/or α_3 receptor subtypes are involved in GABAergic modulation of prolactin secretion.

REFERENCES

1 McKernan RM, Rosahl TW, Reynolds DS et al. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. Nat Neurosci 2000;3(6):587-592.

2 Rudolph U, Crestani F, Benke D et al. Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature 1999;401(6755):796-800.

3 Low K, Crestani F, Keist R et al. Molecular and neuronal substrate for the selective attenuation of anxiety. Science 2000; 290 (5489): 131-134.

4 Rowlett JK, Platt DM, Lelas S, Atack JR, Dawson GR. Different GABA-A receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates. Proc Natl Acad Sci U S A 2005;102(3):915-920.

5 Rudolph U, Crestani F, Mohler H. GABA(A) receptor subtypes: dissecting their pharmacological functions. Trends Pharmacol Sci 2001; 22(4): 188-194.

6 Dias R, Sheppard WF, Fradley RL et al. Evidence for a significant role of alpha 3-containing GABA-A receptors in mediating the anxiolytic effects of benzodiazepines. J Neurosci 2005; 25(46): 10682-10688.

7 Collinson N, Kuenzi FM, Jarolimek W et al. Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABA-A receptor. J Neurosci 2002; 22(13): 5572-5580.

8 Crestani F, Keist R, Fritschy JM et al. Trace fear conditioning involves hippocampal alpha5 GABA(A) receptors. Proc Natl Acad Sci U S A 2002; 99(13): 8980-8985.

9 Rudolph U, Knoflach F. Beyond classical benzodiazepines: novel therapeutic potential of GABA-A receptor subtypes. Nat Rev Drug Discov 2011;10(9):685-697.
10 Tan KR, Brown M, Labouebe G et al. Neural bases for addictive properties of benzodiazepines. Nature 2010;463(7282):769-774.
11 Ben-Jonathan N, Hnasko R. Dopamine as a prolactin (PRL) inhibitor. Endocr Rev 2001; 22(6): 724-763.
12 Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. Physiol Rev 2000; 80(4): 1523-1631.
13 Tappaz ML. GABA and anterior pituitary function: anatomical data. Psychoneuroendocrinology 1984; 9(2): 85-95.
14 Ondo JG, Dom R. The arcuate nucleus: a site for gamma-aminobutyric acid regulation of prolactin secretion. Brain Res 1986; 381(1): 43-48.
15 Lee TY, Pan JT. Involvement of central GABAergic neurons in basal and diurnal changes of tuberoinfundibular dopaminergic neuronal activity and prolactin secretion. Life Sci 2001; 68(17): 1965-1975.
16 Casanueva F, Apud J, Locatelli V et al. Mechanisms subserving the stimulatory and inhibitory components of gamma-aminobutyric acid-ergic control of prolactin secretion in the rat. Endocrinology 1981; 109(2): 567-575.
17 Locatelli V, Cocchi D, Frigerio C et al. Dual gamma-aminobutyric acid control of prolactin secretion in the rat. Endocrinology 1979; 105(3): 778-785.
18 Shin SH, Obonsawin MC, Bates L. Gamma-aminobutyric acid is not likely a physiological prolactin-inhibiting factor. Horm Res 1984;19(1):33-42.
19 AjJouni K, El-Khateeb M. Effect of glucose of growth hormone, prolactin and thyroid-stimulating hormone response to diazepam in normal subjects. Horm Res 1980;13(3):160-164.
20 Laakmann G, Treusch J, Eichmeier A, Schmauss M, Treusch U, Wahlster U. Inhibitory effect of phentolamine on diazepam-induced growth hormone secretion and lack of effect of diazepam on prolactin secretion in man. Psychoneuroendocrinology 1982;7(2-3): 135-139.
21 D'Armiento M, Bisignani G, Reda G. Effect of bromazepam on growth hormone and prolactin secretion in normal subjects. Horm Res 1981; 15(4): 224-227.
22 Beary MD, Lacey JH, Bhat AV. The neuro-endocrine impact of 3-hydroxy-diazepam (temazepam) in women. Psychopharmacology (Berl) 1983; 79(4): 295-297.
23 Zemishlany Z, McQueeney R, Gabriel SM, Davidson M. Neuroendocrine and monoaminergic responses to acute administration of alprazolam in normal subjects. Neuropsychobiology 1990; 23(3): 124-128.
24 Sevy S, Brown SL, Wetzler S et al. Effects of alprazolam on increases in hormonal and anxiety levels induced by meta-chlorophenylpiperazine. Psychiatry Res 1994; 53(3): 219-229.
25 Guldner J, Trachsel L, Kratschmayr C, Rothe B, Holsboer F, Steiger A. Bretazenil modulates sleep EEG and nocturnal hormone secretion in normal men. Psychopharmacology (Berl) 1995; 122(2): 115-121.
26 Copinschi G, Akseki E, Moreno-Reyes R et al. Effects of bedtime administration of zolpidem on circadian and sleep-related hormonal profiles in normal women. Sleep 1995; 18(6): 417-424.
27 Melis GB, Paoletti AM, Mais V et al. The effects of the GABAergic drug, sodium valproate, on prolactin secretion in normal and hyperprolactinemic subjects. J Clin Endocrinol Metab 1982; 54(3): 485-489.
28 Alhambra C, Becker C, Blake T et al. Development and SAR of functionally selective allosteric modulators of GABA-A receptors. Bioorg Med Chem 2011; 19(9): 2927-2938.
29 Zhou D, Sunzel M, Ribadeneira MD et al. A clinical study to assess CYP1A2 and CYP3A4 induction by AZD7325, a selective GABA(A)receptor modulator - an in vitro and in vivo comparison. Br J Clin Pharmacol 2012; 74(1): 98-108.
30 Guo J, Davis PC, Gu C, Grimm SW. Absorption, excretion, and metabolism of a potential GABA-A alpha2/3 receptor modulator in rats. Xenobiotica 2011; 41(5): 385-399.
31 Chen X, Jacobs G, de Kam M et al. The central nervous system effects of the partial GABA-A alpha2,3-selective receptor modulator AZD7325 in comparison with lorazepam in healthy males. Br J Clin Pharmacol 2014; DOI: 10.1111/bcp.12413.
32 Chen X, Jacobs G, de Kam ML et al. AZD6280, a novel partial GABA-A receptor modulator, demonstrates a pharmacodynamically selective effect profile in healthy male volunteers. Submitted 2014.
33 Jacobs GE, Hulskotte EG, de Kam ML et al. Metoclopramide as pharmacological tool to assess VASopressinergic co-activation of the hypothalamus-pituitary-adrenal (HPA) axis: a study in healthy volunteers. Eur Neuropsychopharmacol 2010; 20(12): 866-874.
34 Sassin JF, Frantz AG, Weitzman ED, Kapen S. Human prolactin: 24-hour pattern with increased release during sleep. Science 1972; 177(4055): 1205-1207.
35 Carlson HE, Wasser HL, Levin SR, Wilkins JN. Prolactin stimulation by meals is related to protein content. J Clin Endocrinol Metab 1983; 57(2): 334-338.
36 Quigley ME, Ropert JF, Yen SS. Acute prolactin release triggered by feeding. J Clin Endocrinol Metab 1981; 52(5): 1043-1045.
37 Liem-Moolenaar M, te Beek ET, de Kam ML et al. Central nervous system effects of haloperidol on THC

in healthy male volunteers. J Psychopharmacol 2010; 24(11): 1697-1708.
38 Ashton H. The diagnosis and management of benzodiazepine dependence. Curr Opin Psychiatry 2005; 18(3): 249-255.
39 Ashton H. Guidelines for the rational use of benzodiazepines. When and what to use. Drugs 1994;48(1):25-40.
40 Chouinard G. Issues in the clinical use of benzodiazepines: potency, withdrawal, and rebound. J Clin Psychiatry 2004;65 Suppl 5:7-12.
41 Apud JA, Masotto C, Cocchi D, Locatelli V, Muller EE, Racagni G. Prolactin control by the tubero-infundibular GABAergic system: role of anterior pituitary GABA receptors. Psychoneuroendocrinology 1984;9(2):125-133.
42 Vilkman H, Kajander J, Aalto S et al. The effects of lorazepam on extrastriatal dopamine D(2/3)-receptors - A double-blind randomized placebo-controlled PET study. Psychiatry Res 2009;174(2):130-137.
43 Sieghart W, Sperk G. Subunit composition, distribution and function of GABA(A)receptor subtypes. Curr Top Med Chem 2002; 2(8): 795-816.
44 Crestani F, Martin JR, Mohler H, Rudolph U. Mechanism of action of the hypnotic zolpidem in vivo. Br J Pharmacol 2000; 131(7): 1251-1254.
45 de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JM. Biomarkers for the effects of benzodiazepines in healthy volunteers. Br J Clin Pharmacol 2003; 55(1): 39-50.
46 Chen X, de Haas S, de Kam M, van Gerven J. An overview of the CNS-pharmacodynamic profiles of nonselective and selective GABA agonists. Adv Pharmacol Sci 2012;2012:134523.

Table 1 · Comparison of prolactin levels after administration of lorazepam, AZD7325 and AZD6280 compared with placebo. Treatment differences are expressed as percentages with 95% confidence intervals and p-values.

Treatment comparisons	Percentage difference (95% CI)	p-value
Lorazepam versus placebo	42.0 (31.4/53.5)	<0.0001
AZD7325 2 mg versus placebo	7.6 (-2.8/19.1)	0.1566
AZD7325 10 mg versus placebo	10.5 (-0.2/22.3)	0.0536
AZD6280 10 mg versus placebo	19.8 (8.2/32.6)	0.0007
AZD6280 40 mg versus placebo	32.8 (20.0/47.0)	<0.0001
AZD7325 2 mg versus lorazepam	-24.2 (-31.6/-16.1)	<0.0001
AZD7325 10 mg versus lorazepam	-22.2 (-29.7/-13.9)	<0.0001
AZD6280 10 mg versus lorazepam	-15.7 (-23.8/-6.7)	0.0012
AZD6280 40 mg versus lorazepam	-6.4 (-15.5/3.6)	0.1957

Figure 1 · Time course of plasma concentration of prolactin after administration of single oral doses of 2 mg lorazepam, 2 mg AZD7325, 10 mg AZD7325, 10 mg AZD6280 and 40 mg AZD6280 (AT T = 0 hours). Means are presented with standard errors of the mean (SEM) as error bars.