CHAPTER 7

THE EFFECTS OF THE NONSELECTIVE BENZODIAZEPINE LORAZEPAM AND THE α_2/α_3 SUBUNIT-SELECTIVE $GABA_A$ RECEPTOR MODULATORS AZD7325 AND AZD6280 ON PLASMA PROLACTIN LEVELS

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ABSTRACT

OBJECTIVE: The effect of GABA and GABAergic drugs on prolactin secretion has been evaluated in many studies, often with inconsistent or opposing results. Moreover, little is known about the $GABA_A$ receptor subtypes that could be involved with prolactin release. The present study aimed to provide additional data by evaluating 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.

METHODS: Plasma prolactin concentrations were measured in 32 healthy male volunteers after administration of single oral doses of 2 mg lorazepam, 2 mg or 10 mg AZD7325, 10 mg or 40 mg AZD6280 or placebo.

RESULTS: 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). 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.

CONCLUSIONS: The nonselective benzodiazepine lorazepam and the novel α_2/α_3 subunit-selective $GABA_A$ receptor modulator AZD6280 increase prolactin levels in healthy subjects, 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. Prolactin release following AZD7325 was smaller and 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

Prolactin secretion by the lactotroph cells of the pituitary gland is primarily controlled by the inhibitory influence of dopamine, released predominantly from the hypothalamic tuberoinfundibular dopaminergic neurons[1,2]. A variety of other neurotransmitters, amino acids and neuropeptides have also been demonstrated to influence prolactin secretion. Many of these agents have multiple levels of action, often with opposing effects[2]. The effect of γ-aminobutyric acid (GABA) on prolactin secretion has been evaluated in a large number of in vitro studies, as well as in vivo studies in animals, which have reported both stimulatory and inhibitory effects[3–7]. In contrast, only a few studies have evaluated the direct effects of GABAergic drugs on circulating basal prolactin levels in healthy subjects. Diazepam[8,9] and bromazepam[10] did not significantly affect prolactin levels, while temazepam was found to increase prolactin levels only to a small extent[11]. Alprazolam at high doses increased prolactin levels[12], while lower doses had no significant effect[13]. Zolpidem and bretazenil stimulated nocturnal secretion of prolactin[14,15], while sodium valproate decreased prolactin levels[16]. 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. However, all these studies used small group sizes (6-10 subjects in most studies) and it cannot be excluded that effects might have reached statistical significance if larger study groups had been used.

It has been proposed that GABA exerts a dual control of prolactin secretion, one inhibitory and the other stimulatory[17]. The inhibitory control of prolactin secretion by GABA is thought to occur peripherally at the level of the anterior pituitary. GABA is released from the median eminence into the hypophyseal portal vessels to act at GABA receptors in the anterior pituitary gland[2,18]. Through interaction with GABA receptors on the lactotrophs, GABA inhibits prolactin secretion and also prolactin gene expression[19–21]. However, while there is little doubt that GABA has an inhibitory effect on prolactin release in vitro, a direct in vivo inhibitory effect is less clear[2,22]. The stimulatory control of prolactin secretion by GABA is thought to occur at the level of the central nervous system. GABAergic neurons are present throughout the hypothalamus, including the arcuate nucleus and periventricular area, which constitute the origin of the tuberoinfundibular dopaminergic pathway[23–26]. Within the arcuate nucleus, GABA may directly inhibit the activity of the tuberoinfundibular dopaminergic pathway, with a resulting decrease of pituitary dopamine levels and a concomitant increase in prolactin secretion[2,18,23,27,28].

The counteracting peripheral and central effects of GABA could explain the limited net effect of benzodiazepines on prolactin secretion in healthy volunteers. In addition, although GABA generally causes a reduction of dopaminergic neuronal activity[29], benzodiazepines have also been shown to increase dopamine levels in the mesolimbic pathway[30]. This increase probably results from serial inhibition of coupled GABAergic interneurons in the mesolimbic pathway, which leads to disinhibition of dopaminergic neurons, which outweighs the direct inhibitory influence of benzodiazepines on those dopaminergic neurons[31–33]. Benzodiazepines are allosteric modulators of α_1, α_2, α_3 and α_5 subunit-containing $GABA_A$ receptors[34]. Studies in animal models have provided indications that certain effects of benzodiazepines can be attributed to specific receptor subtypes, such as sedation (α_1 receptor subtype[35,36]), anxiolysis (α_2 and α_3 receptor subtypes[37–40]) and learning and memory (α_5 receptor subtype[41,42]). The disinhibition of mesolimbic dopaminergic neurons and the resulting increase in dopamine levels appear to be mediated by the α_1 receptor subtype[32,33]. It has been suggested that selective agonists at α_3 receptor subtypes without efficacy at α_1 receptor subtypes, may attenuate dopamine neurotransmission in the mesolimbic pathway, without counteractive disinhibition from GABAergic interneurons[31]. Accordingly, $GABA_A$ receptor subunit-selective agonists may differ significantly from nonselective benzodiazepines in their effects on dopaminergic pathways[31].

Little is known about the $GABA_A$ receptor subtypes that could be involved with prolactin secretion. The α_1, α_2, α_3, α_4 and α_5 receptor subtypes are all expressed to some extent in the nucleus accumbens and hypothalamus[43]. To explore the exact role of the various receptor subtypes in the regulation of prolactin secretion, the present study evaluated the effects of two novel α_2/α_3 subunit-selective $GABA_A$ receptor modulators, AZD7325 and AZD6280[44], and a therapeutic dose of lorazepam on prolactin levels in an adequately powered group of 32 healthy volunteers. In vitro receptor binding studies demonstrated that AZD7325 and AZD6280 have high affinity for the α_1 receptor subtype (mean $K_i = 0.5$ nM for both compounds), α_2 receptor subtype (mean $K_i = 0.3$ and 21 nM, respectively) and α_3 receptor subtype (mean $K_i = 1.3$ and 31 nM, respectively), and lower affinity for the α_5 receptor subtype (mean $K_i = 230$ and 1680 nM, respectively) (AstraZeneca data on file). Electrophysiological assays to evaluate the potentiation of GABA-induced current relative to the maximal response of diazepam (set at 100%) demonstrated potentiation by AZD7325 and AZD6280 at the α_2 receptor subtype (18% and 32%, respectively) and α_3 receptor subtype (15% and 34%, respectively), but neutral antagonism at the α_1 receptor subtype (6% and 8%, respectively) and α_5 receptor subtype (8% and 7%, respectively) (Astra-

Zeneca data on file). These preclinical data suggest that AZD7325 and AZD6280 are selective modulators of α_2 and α_3 subunit-containing $GABA_A$ receptors. Based on positron emission tomography (PET) using [^{11}C]flumazenil in healthy volunteers, AZD7325 at 2 and 10 mg doses and AZD6280 at 10 and 40 mg doses are expected to result in substantial occupancy of $GABA_A$ receptors in the brain (e.g., ›50% occupancy of the maximal displaceable [^{11}C]flumazenil binding in occipital cortex) (AstraZeneca data on file). Previous studies have demonstrated that sleep-inducing doses of classical benzodiazepines such as clonazepam[45], diazepam[46], midazolam[47] and lorazepam[48-50], as well as the nonbenzodiazepine GABA receptor agonist zolpidem[51] are associated with relatively low receptor occupancy levels (up to approximately 30%), whereas pharmacologically active doses of the α_2/α_3 subunit-selective $GABA_A$ partial agonists TPA023[48] and TPA023B[52] are associated with higher occupancy levels (i.e. approximately 50%). Also, doses of 2 and 10 mg of AZD7325 and 10 and 40 mg of AZD6280 are predicted to lead to peak plasma concentrations above minimally efficacious concentrations in animal models of anxiety (AstraZeneca data on file). These data demonstrate that AZD7325 and AZD6280 cross the blood-brain barrier, interact with the target receptor, and have the potential to produce anxiolytic activity in humans.

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/m^2 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[53] 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 lorazepam and prolactin

Venous blood samples for analysis of lorazepam and 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 lorazepam were determined using LC-MS/MS with a lower limit of quantification of 0.3 ng/mL. Plasma concentrations of AZD7325 were determined using LC-MS/MS with a lower limit of quantification of 0.05 ng/mL. Plasma concentrations of AZD6280 were determined using LC-MS/MS with a lower limit of quantification of 0.15 ng/mL. Plasma concentrations of prolactin were determined using an electrochemiluminescence immunoassay (ECLIA) with a lower detection limit of 0.047 ng/mL.

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 lorazepam, AZD7325 and AZD6280

Plasma concentrations of lorazepam, AZD7325 and AZD6280 are shown in Figures 1, 2 and 3, respectively. The pharmacokinetics of lorazepam were similar to those reported in literature[54].

FIGURE 1 Time course of plasma concentration of lorazepam after administration of single oral doses of 2 mg lorazepam (at t = 0 hours). Means are presented with standard deviations as error bars.

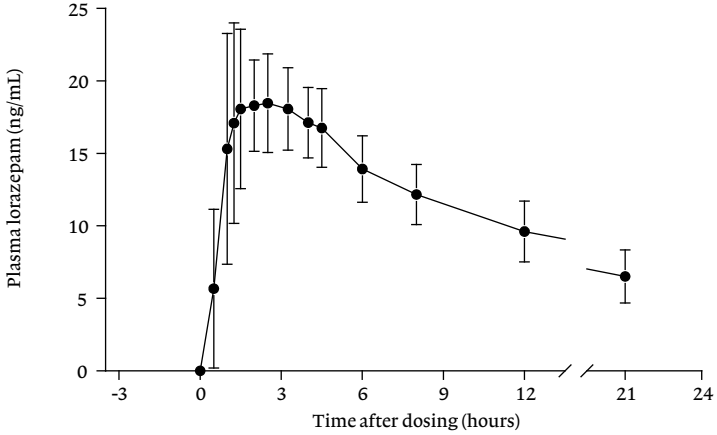

FIGURE 2 Time course of plasma concentration of AZD7325 after administration of single oral doses of 2 mg and 10 mg AZD7325 (at t = 0 hours). Means are presented with standard deviations as error bars.

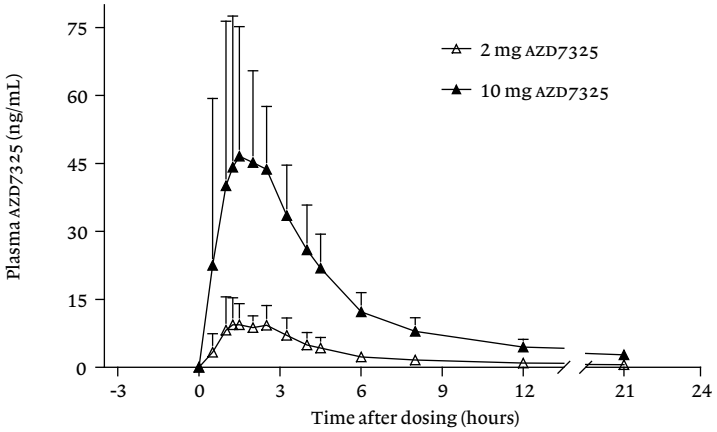

FIGURE 3 Time course of plasma concentration of AZD6280 after administration of single oral doses of 10 mg and 40 mg AZD6280 (at t = 0 hours). Means are presented with standard deviations as error bars.

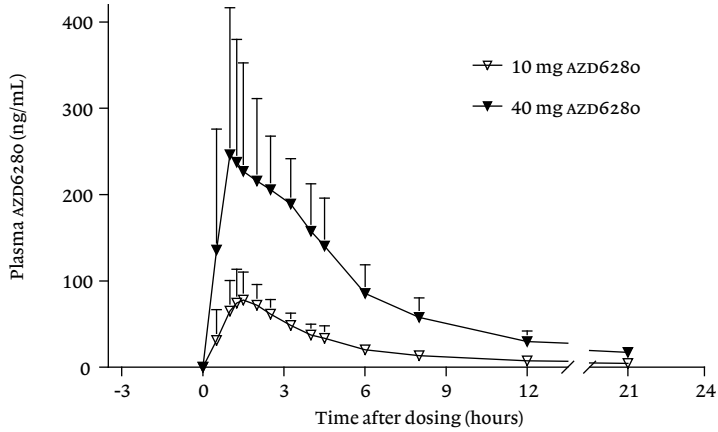

FIGURE 4 Time course of plasma concentration of prolactin after administration of single oral doses of 2 mg lorazepam, 2 mg AZD7325, 10 mg AZD7325, 10 mg AZD6280 and 40 mg AZD6280 (at t = 0 hours). Means are presented with standard deviations as error bars.

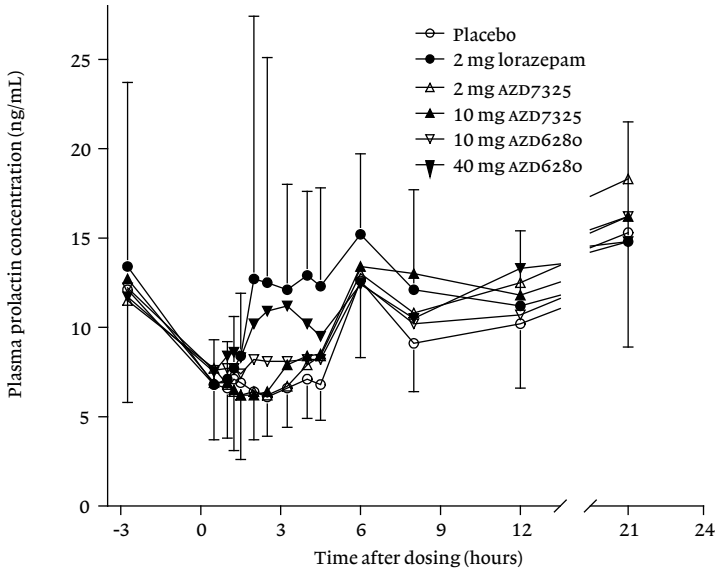

TABLE 1 Comparison of prolactin levels after administration of lorazepam, AZD7325 and AZD6280 compared with placebo. Treatment differences are expressed as percentages with 95% confidence intervals and p-values.

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

Plasma concentration of prolactin

Plasma concentrations of prolactin after administration of lorazepam, AZD7325 and AZD6280 are shown in Figure 4 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

The present study was performed to evaluate the effects of the GABAergic drugs on circulating prolactin levels in healthy subjects, compared with 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[2,55]. Also, a peak in prolactin levels was observed 6 hours

after dose administration, which probably reflects normal prolactin release following food consumption[56,57]. 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%[58]. Thus, the effects of lorazepam administration on prolactin secretion are not likely to produce clinically relevant hyperprolactinaemia. 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[8–10], 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[11]. 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[59–61]. 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)[12], while doses in the lower therapeutic range (0.5 mg) demonstrated no effects[13]. 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 (either directly at the anterior pituitary gland or by stimulating GABA release from the median eminence into the hypophyseal portal vessels). 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[62], 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[63], 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 also 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 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[64], increased nocturnal prolactin levels by two-fold[15]. Furthermore, despite functional selectivity for α_2 and α_3 receptor subtypes, AZD7325 and AZD6280 also have limited efficacy at α_1 receptor subtypes (AstraZeneca data on file).

The effects of AZD7325 on prolactin secretion were less clear than those of AZD6280. Similarly, in other studies (Chen et al 2013, submitted; Chen et al 2013, in preparation), 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[65] and α_2/α_3 subtype-selective compounds[66] in healthy volunteers. These differences may be related to the lower dosages of AZD7325 used. Although the dosages of both compounds were expected to result in relatively high levels of $GABA_A$ receptor occupancy, AZD7325 is less effective in potentiation of GABA-induced current than AZD6280 at the α_2 receptor subtype (18% and 32%, respectively, compared to the maximal response of diazepam) and α_3 receptor subtype (15% and 34%, respectively), whereas efficacy is very low and roughly similar at the α_1 and α_5 receptor subtypes (AstraZeneca data on file).

In conclusion, our study demonstrates that single oral doses of 2 mg lorazepam increase plasma prolactin levels in healthy male subjects. Our findings contrast with the inconsistent results obtained in earlier studies with other GABAergic drugs. The sample sizes or the administered doses used in these earlier studies may have been too small to adequately demonstrate statistically significant differences, whereas our present results were obtained in an adequately powered group of 32 healthy volunteers, although it is not excluded that the different outcomes are caused by pharmacological differences between the various drugs. Our study also evaluated the effects of two novel α_2/α_3 subunit-selective $GABA_A$ receptor modulators, AZD7325 and AZD6280, on prolactin levels. AZD6280 produced significant increases in prolactin levels, which may indicate that the α_2 and/or α_3 receptor subtypes are involved in GABAergic modulation of prolactin secretion, although contributions of the α_1 and α_5 receptor subtypes are not excluded. The increases in prolactin levels after administration of AZD7325 did not reach statistical significance, which may be related to the lower dosages of AZD7325 used.

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