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Acute benzodiazepine administration induces changes in homocysteine metabolism in young healthy volunteers

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ABSTRACT

Purpose: High cortisol plasma concentrations have been shown to be associated with increases in homocysteine levels. Here we studied whether decreases in cortisol concentration, induced by an acute oral dose of a benzodiazepine, could decrease homocysteine, and if changes were similar in both genders.

Methods: This was a double-blind, cross-over design study of acute oral flunitrazepam (1.2 mg) and placebo in young, healthy, male and female ($n=21$) volunteers. Blood samples were collected 3 h after ingestion (after peak-plasma concentration of flunitrazepam was reached). Various biochemical parameters were analysed, such as plasma homocysteine, cysteine, folate, vitamins B6, B12, and sexual hormones.

Results: Flunitrazepam reduced cortisol ($p=0.0011$), cysteine ($p=0.014$) and homocysteine ($p=0.028$) concentrations, irrespective of gender. No correlations were found between cortisol and other biochemical markers (all $r^2<0.03$). Concentration of cysteine and homocysteine were negatively correlated with plasma flunitrazepam concentration, suggesting that changes in these amino acids might be related to the metabolism of this benzodiazepine.

Conclusion: Acute administration of flunitrazepam decreases plasma homocysteine and cysteine by mechanisms that seem unrelated to changes in cortisol. Given the importance of homocysteine as a marker of life-threatening disorders, the mechanisms involved in the decrease of these amino acids are potential targets for clinical application.

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1. Introduction

Homocysteine is a sulphur intermediate amino acid in methionine-cysteine metabolism (Finkelstein and Martin, 2000). Increases in total homocysteine plasma concentrations have been linked experimentally and epidemiologically with neurological and psychiatric disorders (e.g. Obeid et al., 2007; Refsum et al., 2004), and serve as an indicator of cardiovascular disease (see Myers et al., 2008; but cf. Moat, 2008). In addition, anxiety symptoms have been found to be associated with increased homocysteine concentration in healthy adults, irrespective of various potential confounders (Pitsavos et al., 2006), but the mechanisms underlying the relation between homocysteine and anxiety are still unresolved. One possibility is that it relates to changes in cortisol concentrations, which is increased in many disorders and also by states

related to anxiety (see Erickson et al., 2003). Patients with Cushing's syndrome, characterized by excessive cortisol production, also exhibit hyperhomocysteinemia (Faggiano et al., 2005; Terzolo et al., 2004). Faggiano et al. (2005) have suggested that the increase of cortisol in these patients could elevate homocysteine by impairment of the remethylation of homocysteine to methionine, or by a stimulation of the trans-methylation from methionine to homocysteine. If these mechanisms do exist, reductions in cortisol could elicit a decrease in homocysteine. The objective of the present study was to evaluate this hypothesis through the administration of an acute oral dose of a benzodiazepine, a class of drugs that are effective anti-anxiety agents which also acutely decrease cortisol concentration (see Arvat et al., 2002; Breier et al., 1992; Hommer et al., 1986; Kalogeras et al., 1990; Risby et al., 1989; Rohrer et al., 1994; Zemishlany et al., 1990), even in non-anxious volunteers.

We also studied whether men and women differ in their response to decreases in cortisol after acute administration of benzodiazepines, and potential alterations of homocysteine that may follow. The reason for this is that people of both genders are not equivalent when it comes to measures of cortisol and homocysteine (see Tallova et al., 1999; Jacobsen et al., 1994; Kajantie and Philips, 2006; Selhub, 1999; Smolders et al.,

Abbreviations: LH, luteinizing hormone; FSH, follicle stimulating hormone; SHBG, sex hormone binding globulin; T3, triiodothyronine; T4, thyroxine; T, testosterone; HPLC, High performance liquid chromatography; HSD, Tukey's Honest Significant differences test; ANOVA, Analysis of variance.

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2003) although they do not differ in terms of other biochemical markers in homocysteine metabolism such as folate, vitamins B12 and B6 (Sassi, 2002).

In order to decrease cortisol we employed the benzodiazepine flunitrazepam, one of the compounds with highest affinity to the benzodiazepine receptors (Mattila and Larni, 1980; Mohler and Okada, 1977) which was used in a dose (1.2 mg) a little larger than the standard 1 mg clinical dose to ensure measurable effects. Normal, non-anxious volunteers were evaluated because stressed and/or anxious individuals can present a myriad of physiological alterations (e.g. increases in catecholamines) (Erickson et al., 2003) that could act as confounders in establishing the relations between cortisol and homocysteine. Because of the alterations of both cortisol (Kajantie and Phillips, 2006) and homocysteine (Tallova et al., 1999) during different phases of the menstrual cycle, and also because hormonal fluctuations elicit physiological alterations that can change the pharmacokinetics of various drugs (Gandhi et al., 2004), the female volunteers in the present study were taking a standardized oral contraceptive that has been shown not to influence homocysteine plasma concentrations (Lussana et al., 2003). Also, men and women do not seem to differ in terms of benzodiazepine metabolism (Greenblatt et al., 2004; Greenblatt and Wright, 1993; Kashuba et al., 1998; Kharasch et al., 1999), including flunitrazepam (Jochimsen et al., 1983), even when benzodiazepines are combined with oral contraceptives (Ochs et al., 1987). In addition to measures of plasma cortisol and homocysteine, we measured changes in subjective anxiety and sedation, as well as of other biochemical factors such as those in homocysteine metabolism (cysteine, folate, vitamin B6 and B12) and male and female hormones, including those in women's contraceptive pill.

2. Methods

2.1. Participants

Participants were 24 (12 men), 18–35 years-old healthy volunteers, with normal body mass index, who did not smoke and were not heavy alcohol drinkers. Subjects did not report psychiatric disorders, including drug abuse, or clinical disorders that could have influence the effects of flunitrazepam, such as epilepsy, diabetes, kidney, hepatic or hormonal disorders, and effects of contraceptive pill and measures of homocysteine metabolism, such as personal or family history of cardiovascular disease. They had normal trait anxiety and depression scores as assessed by the adapted versions to the Brazilian population of the State-Trait Anxiety Inventory and the Beck Depression Inventory (Gorenstein and Andrade, 1996). Participants were taking no psychotropic drugs or hormones (except the oral contraceptives, see below) at the time of the study. Women were taking an oral contraceptive containing 0.075 mg of gestodene and 0.02 mg of ethinylestradiol for at least a month prior to the study, an oral contraceptive that seems to minimally alter metabolism of benzodiazepines (Palovaara et al., 2000). Women who were pregnant, breast-feeding, or who had used other hormonal contraceptives in the previous 6 months were excluded from the study.

2.2. Procedures

The Ethics Committee of the institution (Universidade Federal de São Paulo–UNIFESP) approved the protocol which was conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent. This was a double-blind, cross-over, placebo-controlled design study of single oral doses of flunitrazepam (1.2 mg) and placebo (lactose) involving two experimental sessions separated by at least 4 days. Women were evaluated between 10th and 21st day of cycle when the exogenous hormone concentrations reach a relatively stable concentration (Dibbelt et al., 1991, 1992). All female volunteers took the oral contraceptive in the morning (prior to the

testing session) in the month of the experiment, except for one who did not agree to change her usual night pill taking schedule. Order of treatments was random and equivalent in both genders. The volunteers were instructed to abstain from alcohol or other drugs for 24 h before and after the experiment. They were administered the drug in the morning after a light breakfast. Peak-plasma concentration in healthy subjects after oral administration of this compound is reached after 1.3 h (ranging from 0.3 to 3 h) and elimination half-life is 35 h (15–66 h) (Jochimsen et al., 1983), so blood collection was conducted around 3 h after oral administration—with at least 4 h of fasting—to ensure that maximum effects could be found for all volunteers. Visual-analogue mood scales were completed to measure subjective anxiety and sedation levels after treatments, just before blood collection. Between peak-plasma concentration and blood collection subject underwent cognitive tasks (results will be published elsewhere).

2.3. Treatments

Treatments were formulated in identical capsules containing 1.2 mg of flunitrazepam plus lactose (flunitrazepam treatment) or lactose only (placebo treatment).

2.4. Biochemistry analysis

We analyzed plasma concentration of homocysteine, cysteine, vitamins B6 and B12, folate, cortisol, estradiol, prolactin, progesterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG), total and free fractions of triiodothyronine (T3), thyroxine (T4) and testosterone (T), and the hormones contained in the oral contraceptive in the blood sample withdrawn from women only—gestodene and ethinylestradiol. Blood samples were obtained in a standard form considering homocysteine metabolism markers and were left at room temperature for a maximum of 30 min before obtaining plasma. Homocysteine and cysteine values were determined in plasma (EDTA) by high performance liquid chromatography (HPLC; Shimadzu, Kyoto, Japan) with fluorimetric detection and isocratic elution (Pfeiffer et al., 1999). Vitamin B6 was quantified in serum using HPLC with ultraviolet detection and isocratic elution (Sharma and Dakshinamurti, 1992). Serum concentration of vitamin B12, folate, cortisol, estradiol, prolactin, progesterone, LH, FSH, SHBG, total and free T3 and T4 and total testosterone were measured by chemiluminescent assay kits (IMMULITE 2000). Free testosterone was measured by a solid phase radioimmunoassay using a commercial assay (Coat-A-Count; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). The concentrations of gestodene, ethinylestradiol and flunitrazepam in human plasma were determined by using validated methods of high performance liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS).

2.5. Visual-analogue mood scales

In order to measure subjective feelings of levels of *sedation* and *calm*, subjects were instructed to indicate how they felt just before blood collection by marking points on 100 mm horizontal lines representing the full range of these dimensions (see Bond and Lader, 1974). Higher score indicated more sedation and less calm.

2.6. Statistical analysis

Plasma concentration of the hormones were submitted to two-way analysis of variance (ANOVAs) with treatment (flunitrazepam or placebo) and gender as factors, followed by Tukey's Honest Significant Differences (HSD) tests when applicable. We also used ANOVAs with treatment and order (placebo or flunitrazepam first) as factors. Pearson product moment correlations were calculated between

flunitrazepam (drug session only) and homocysteine concentration with the remaining biochemical parameters. Effect sizes (see Cohen, 1988; Fredrickson et al., 2008; Morris and DeShon, 2002; Snyder et al., 2005) were calculated to determine the magnitude of flunitrazepam-induced changes in the homocysteine and cysteine concentrations. The level of significance adopted was 5%. Data not shown below did not reach statistical significance.

3. Results

LH data of one male subject was excluded from the analysis due to layout values at both sessions. Samples from 3 female volunteers were not obtained after two failed attempts to withdraw blood. Hence, our final sample as concerns biochemical analyses consisted of 12 males (except for the LH measure which included 11 measures) and 9 females.

Demographic characteristics: subjects of both genders were equivalent in terms of mean \pm SD age (men 23.0 ± 2.7 ; women 23.8 ± 2.0 ; $p > 0.37$) and body mass index (kg/m^2 : men 23.4 ± 1.5 ; women 22.6 ± 2.4 ; $p > 0.37$), but women weighed (59.8 ± 9.9 kg) less than men (76.9 ± 7.0 kg) ($F_{1,22} = 24.17$, $p < 0.0001$) and men were taller than women (men 1.82 ± 0.09 m; women 1.62 ± 0.07 m) ($F_{1,22} = 35.79$, $p < 0.0001$). The mean pill cycle day, as well as the concentrations of gestodene and ethinylestradiol did not differ between women in both experimental sessions ($p > 0.18$).

Biochemistry analysis (Table 1): only simple effects of treatment and gender were observed. There were no interactions, indicating that the alterations brought about by flunitrazepam were equivalent in both men and women, even in cortisol and homocysteine concentrations, which are known to differ between genders. Gender effects in general reflected use of the contraceptive pill by the females, who had higher SHBG plasma concentrations than men ($F_{1,19} = 665.15$, $p < 0.0001$), while the opposite occurred for progesterone ($F_{1,19} = 4.55$, $p = 0.046$), estradiol ($F_{1,19} = 25.86$, $p < 0.0001$), total ($F_{1,19} = 171.96$, $p < 0.0001$) and free ($F_{1,19} = 175.78$, $p < 0.0001$) testosterone, vitamin B6 ($F_{1,19} = 5.17$, $p = 0.036$), and B12 ($F_{1,19} = 5.93$, $p = 0.025$). Flunitrazepam reduced cortisol in both genders ($F_{1,19} = 14.63$, $p = 0.0011$), despite measures having been lower in men than women ($F_{1,19} = 5.26$, $p = 0.033$). This drug-treatment also reduced prolactin ($F_{1,19} = 11.77$, $p = 0.0028$), as well as both cysteine ($F_{1,19} = 5.64$, $p = 0.028$) and homocysteine ($F_{1,19} = 7.26$, $p = 0.014$), arguing against a type I error since these are both amino-acids in the same metabolic system. For LH, higher concentrations were observed in men ($F_{1,19} = 28.56$, $p < 0.0001$), and after treatment with

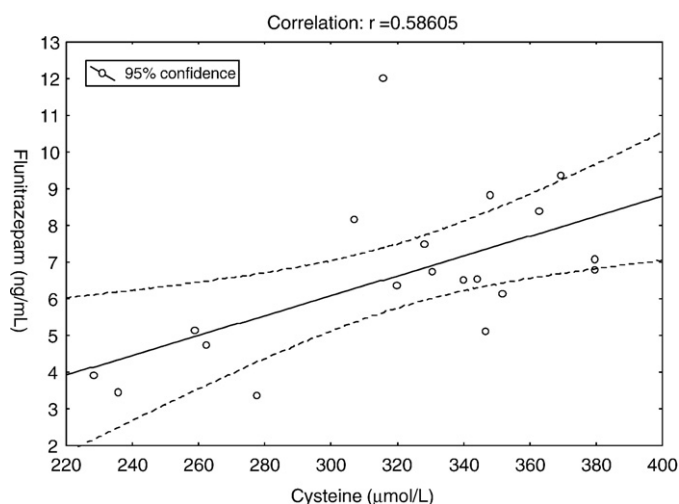


Fig. 1. Pearson product moment correlations between plasma cysteine and flunitrazepam concentrations ($p < 0.009$).

flunitrazepam ($F_{2,38} = 4.35$, $p = 0.05$). For total T3 ($F_{1,19} = 35.16$, $p < 0.0001$) and T4 ($F_{1,19} = 51.87$, $p < 0.0001$), but not free T3 and T4 fractions which have biological activity, samples from women had higher concentrations.

Because of the reduced sample size and the small numerical difference in the mean decrease of both homocysteine and cysteine concentration, we computed effect size calculations in order to determine the magnitude of these effects. To this end we used the mean and standard-deviations of the concentration of homocysteine and cysteine after the two treatment manipulations (placebo, drug) including all individuals, since there was no gender effect in the measures for both amino acids. We also considered the correlation between the measures after placebo and flunitrazepam because we used a cross-over design (see Morris and DeShon, 2002, Eq. 8). Effect size change for homocysteine was $d = 0.559$ and for cysteine $d = 0.502$. Both these values indicate moderate effects sizes (Cohen, 1988), which are considered clinically relevant.

Cortisol concentration failed to correlate significantly with any measures in both placebo- and flunitrazepam-treated volunteers (all r values < 0.03 and p values > 0.47). However, significant correlations were found between homocysteine and cysteine in the placebo

Table 1

Mean (\pm SD) values for each gender and treatments in the blood sample variables, as well as day of the menstrual cycle and exogenous hormone concentrations in women only.

	Effect*	Placebo		Flunitrazepam	
		Men	Women	Men	Women
Triiodothyronine (T3) (ng/dl)	G	130 \pm 30	202 \pm 37	119 \pm 24	196 \pm 30
Thyroxine (T4) ($\mu\text{g}/\text{dL}$)	G	7.9 \pm 1.5	11.5 \pm 1.3	7.6 \pm 1.6	11.3 \pm 2.0
Free T3 (pg/dL)		3.3 \pm 0.4	3.2 \pm 0.4	3.3 \pm 0.4	3.2 \pm 0.4
Free T4 (ng/dL)		1.3 \pm 0.1	1.3 \pm 0.2	1.3 \pm 0.2	1.3 \pm 0.1
Luteinizing hormone (LH) (mIU/ml)	G; T	5.6 \pm 2.8	0.3 \pm 0.3	7.5 \pm 4.5	0.3 \pm 0.5
Follicle stimulating hormone (FSH) (mIU/mL)		5.9 \pm 9.0	1.2 \pm 1.0	6.7 \pm 11.2	1.2 \pm 1.0
Prolactin (ng/mL)	T	7.4 \pm 3.2	7.0 \pm 2.5	6.2 \pm 2.8	5.3 \pm 1.7
Progesterone (ng/mL)	G	0.7 \pm 0.2	0.6 \pm 0.4	0.8 \pm 0.2	0.5 \pm 0.3
Estradiol (pg/mL)	G	29.8 \pm 9.5	17.4 \pm 7.7	27.7 \pm 9.0	11.0 \pm 2.3
Total testosterone (ng/dL)	G	553 \pm 148	35 \pm 11	562 \pm 135	29 \pm 12
Free testosterone (pg/mL)	G	17.5 \pm 3.7	0.2 \pm 0.1	17.7 \pm 4.5	0.2 \pm 0.1
Cortisol ($\mu\text{g}/\text{dL}$)	G; T	10.5 \pm 2.1	13.5 \pm 4.4	6.5 \pm 4.7	9.6 \pm 3.3
Sex hormone binding globulin (SHBG) (nmol/L)	G	21.5 \pm 7.9	162.6 \pm 26.2	21.2 \pm 6.0	164.4 \pm 23.3
Homocysteine ($\mu\text{mol}/\text{L}$)	T	12.1 \pm 2.4	11.6 \pm 2.3	11.3 \pm 2.4	10.2 \pm 2.2
Cysteine ($\mu\text{mol}/\text{L}$)	T	335 \pm 40	343 \pm 43	319 \pm 52	309 \pm 41
Vitamin B6 (nmol/L)	G	32.8 \pm 8.8	24.1 \pm 7.7	33.0 \pm 5.9	25.7 \pm 7.9
Vitamin B12 (pg/mL)	G	409 \pm 107	279 \pm 61	408 \pm 128	302 \pm 138
Folate (ng/mL)		7.5 \pm 5.5	10.0 \pm 4.4	8.8 \pm 5.5	8.6 \pm 5.1
Day of menstrual cycle			14.9 \pm 4.2		14.0 \pm 4.1
Ethinyl estradiol (pg/mL)			43.6 \pm 22.0		38.7 \pm 21.7
Gestodene (pg/mL)			7517 \pm 2207		7074 \pm 2684

NB: *ANOVA G = gender effects and/or T = treatment effects ($p < 0.05$). No interaction between gender and treatment were found.

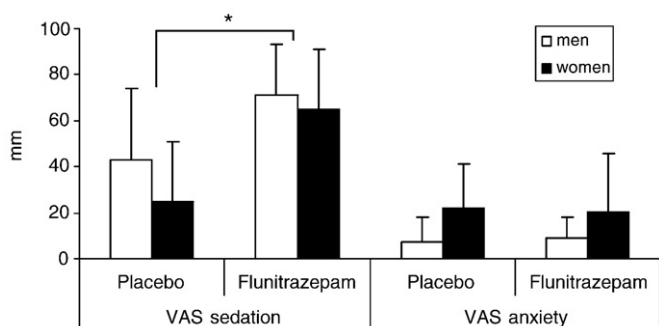


Fig. 2. Mean (\pm SD) of visual-analogue mood scales (VAS) of subjective sedation and anxiety levels per treatment and gender. *Flunitrazepam increased sedation, irrespective of gender ($p < 0.05$), but did not alter feelings of “calm” in both men and women.

($r = 0.74$) and flunitrazepam treatments ($r = 0.92$) (p 's < 0.0001), and between flunitrazepam plasma concentration and cysteine ($r = 0.59$; $p = 0.009$; Fig. 1), and a trend for homocysteine ($r = 0.41$; $p = 0.08$).

For the visual-analogue scales (Fig. 2), no effect of drug or gender was found in reports of how calm participants were, despite women having tended to rate themselves as slightly less calm than men, irrespective of the treatment session ($F_{1,21} = 36.95$, $p < 0.07$). In any case, ratings after flunitrazepam and placebo in both genders were set near the extremity of the scale that indicated that subjects felt calm. Treatment with flunitrazepam, however, led to higher ratings of sedation than after placebo, irrespective of gender ($F_{1,21} = 18.00$, $p = 0.0006$), which would be expected for a hypnotic drug.

4. Discussion

Plasma concentration of both homocysteine and cysteine decreased significantly after ingestion of flunitrazepam in both men and women, suggesting that this drug, either directly or indirectly, alters the homocysteine metabolic pathway irrespective of gender in young, healthy individuals in whom homocysteine metabolism was preserved and who had no nutritional deficiencies, as confirmed by normal ranges of concentrations of folate, vitamins B6 and B12. These statistical findings were further confirmed by effect size calculations which rendered clinically relevant magnitudes (see Cohen, 1988).

One possible explanation for the decrease in homocysteine and cysteine concentration brought about by flunitrazepam is that it derived from the fall in cortisol levels, an inhibitory effect on HPA axis that has been shown after ingestion of other benzodiazepines (Breier et al., 1992; Hommer et al., 1986; Kalogeras et al., 1990; Risby et al., 1989; Rohrer et al., 1994). Since glucocorticoid excess has been linked to homocysteine metabolic abnormalities that raise homocysteine plasma concentration (Faggiano et al., 2005), it could therefore follow that a decrease in cortisol could have the opposite effect. However, the lack of correlation between cortisol and homocysteine and cysteine after the flunitrazepam treatment argues against this hypothesis and confirms the lack of consistent effects of changes in glucocorticoid concentrations on homocysteine metabolism (see Berg et al., 2006). In effect, the administration of glucocorticoids to young, healthy subjects for a few days led to no changes in homocysteine (Berg et al., 2006; Brotman et al., 2005).

The lack of changes in folate, vitamins B6 and B12 also do not support the idea that the flunitrazepam-induced decreases in cysteine and homocysteine are related to the metabolic pathway of these amino acids. We can suggest two explanations for the correlations between flunitrazepam plasma concentration and cysteine, and the trend in correlation between this benzodiazepine and homocysteine. First, the metabolism of this benzodiazepine may have exerted its effects on cysteine, and consequently on homocysteine concentrations, since for

cysteine biotransformation many endogenous species are used as acceptors for the benzodiazepine functional group during cytochrome P450 processing (Coon, 2003). Alternatively, the benzodiazepine-induced changes in cysteine and homocysteine could result from glutathione depletion. The depletion of glutathione, which is always associated with cytochrome P450 enzymes activity involved in benzodiazepine metabolism, could lead to cysteine consumption for the synthesis of glutathione (Halliwell and Gutteridge, 2007).

Further experimentation is needed to investigate these hypotheses and to elucidate the seemingly cortisol-independent mechanism of homocysteine metabolism changes in these young, healthy participants after acute ingestion of a benzodiazepine, especially as the sample size of the present study was small and homocysteine metabolism is influenced by many factors (e.g. Refsum et al., 2004), including diet (Bidulescu et al., 2009; Mourvaki et al., 2005), which was not controlled in the present study. Blood pressure is also an important factor (Bidulescu et al., 2009; Mourvaki et al., 2005) that was not measured in the present study, although it is unlikely to have influenced results because volunteers with personal or family history of cardiovascular disease were not included in the sample.

The replication of our results may lead to the development of treatment for patients with faulty homocysteine metabolism. If, for instance, these mechanisms are found to be effective after repeated dosing of benzodiazepines, and it is taken into account that homocysteine (e.g. Bleich et al., 2006), and prolactin (Hillemecher et al., 2007), which was also reduced after flunitrazepam, have a relatively high potential to induce seizures, our finding may lend further support to the suggestion that benzodiazepines be prescribed as anticonvulsants in clinical practice in treatment, for instance, of alcohol withdrawal in hyperhomocysteinemia patients (see Bleich et al., 2004).

The remaining hormone concentrations evaluated here did not correlate with cortisol, homocysteine nor flunitrazepam plasma concentration, suggesting that they are not related to changes in the homocysteine metabolic pathway. The decrease in prolactin induced by flunitrazepam can relate to high levels of sedation (Jarvinen et al., 1992) that was found here, and has been shown for other benzodiazepine (Boydjjeva et al., 1986; Grandison, 1982), as has the increase in LH (Thomas et al., 1989). Also, lack of change in subjective self-rated levels of anxiety confirms results of Hommer et al. (1986) who administered diazepam to non-anxious participants.

The gender effect findings also reflect previously known phenomena and did not seem related to the changes in the homocysteine and cysteine values. Oral contraceptive intake explains female's lower concentrations of LH (Hemrika et al., 1993), free and total testosterone (Coenen et al., 1996), progesterone and estradiol (Endrikat et al., 2004), as well as decreases in vitamin B6 and B12 (Rohrer et al., 1994). Also, higher T3 and T4 total plasma concentration in women are related to increases in SHBG due to the ingestion of exogenous hormones (Coenen et al., 1996; Wiegatz et al., 2003). However, the free portions of T3 and T4, which have biological effect through interaction with their receptors, were unchanged by flunitrazepam, so the differences in the total plasma concentrations of T3 and T4 are unlikely to elicit distinct effects in men and women.

It has been recently described that homocysteine competitively binds to and inhibits GABA_A receptors (see Tyagi et al., 2007). Also, Tsvetnitsky et al. (1995) showed that both S-adenosylhomocysteine and 5'-methylthioadenosine, which are steps in homocysteine metabolism, are able to inhibit flunitrazepam binding to benzodiazepine receptors, suggesting that these compounds may be candidate endogenous benzodiazepine receptor ligands. Our experimental data showed that the administration of flunitrazepam reduced plasma homocysteine concentrations, so a question that arises when considering these results is the possible interaction between benzodiazepines and homocysteine at the GABA_A /benzodiazepine receptors. The existence of a mechanism by which the presence of benzodiazepine ligands, whether exogenous or not, may lead to reductions in endogenous homocysteine plasma

concentration would enable these drugs to have larger inhibitory effects at their receptor sites.

5. Conclusion

The acute oral administration of a benzodiazepine decreased plasma concentration of homocysteine and cysteine through an as yet undetermined mechanism that may relate to the metabolism of flunitrazepam, but that did not seem related to the flunitrazepam-induced decrease in cortisol. Given the importance of homocysteine and cysteine as risk factors to various seriously debilitating psychiatric and, at times, fatal disorders, the elucidation of the mechanisms by which benzodiazepines decrease these amino acids may have important therapeutic potential.

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