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Are Women Sensitive to the Acute Anxiolytic Effect of Diazepam?

Camila Danielle Aragão-Almeida, Fabrício Dias Antunes, Vânia dos Santos Barbosa and Flavia Teixeira-Silva*

Departamento de Fisiologia, Centro de Ciências Biológicas e da Saúde, Universidade Federal de Sergipe, Cidade Universitária 'Prof. José Aloísio de Campos', 49100-000, Sergipe, Brazil

Abstract

Objective: Several studies have shown the influence of ovarian hormones on the GABAergic system. As women are naturally exposed to monthly fluctuation of these hormones, it is possible that their response to benzodiazepines also change over the ovarian cycle. Bearing this in mind, this study aimed to evaluate the possible influence of the ovarian cycle of healthy women on the acute effect of diazepam.

Methods: Forty subjectively healthy women were selected and randomly allocated to two different groups, according to their ovarian cycle phase, follicular (6 to 10 days from the first day of the cycle) or luteal (5 to 10 days after detection of urinary LH peak). Both groups completed the Video-Monitored Stroop Color-Word Test (VMSCWT), an experimental model of anxiety, under the influence of diazepam (10 mg) or placebo. Psychological parameters (State-Trait Anxiety Inventory, Self-evaluation of Tension Level, Visual Analogue Mood Scale) and physiological parameters (heart rate and gastrocnemius electromyogram activity) were evaluated throughout the test. All the data obtained were analyzed using analysis of variance (ANOVA) followed by Tukey's test for post hoc comparisons, both at the 5% significance level.

Results: The results showed that, in the follicular phase, women did not respond to the anxiolytic action of diazepam, although a sedative effect was observed; while in the luteal phase, there was no response to either sedative or anxiolytic actions. As a control for the experimental conditions, a group of 18 men was also administered to the VMSCWT. The results confirmed that both the anxiogenic test and the administered drug were working as expected, since diazepam managed to prevent the anxiety induced by the test.

Conclusion: Therefore, the present findings indicate that the ovarian cycle can alter the effects of the acute administration of diazepam, which can vary from no effect to sedation, without going through anxiolysis.

Keywords: Diazepam; Ovarian cycle; Experimentally-induced anxiety; Stroop test; Healthy subjects; Female brain; Sex hormones

Introduction

The ovarian hormones as all steroid hormones are synthesized from cholesterol, and due to its lipophilicity, have easy access to all cells and organs, including the central nervous system (CNS) [1]. At the cerebral level, these hormones influence the function of many nervous cells, playing an important role in coordinating a number of physical and behavioral changes related to reproductive cycle [2]. However, studies are highlighting the critical role that ovarian hormones may have on the organization of non-reproductive behavior, especially in response to stress and anxiety [3-6].

Progesterone, in the corpus luteum, is converted into, among other metabolites, allopregnanolone (3α-hydroxy-5α-pregnan-20-one), which is secreted under stimulation of the luteinizing hormone (LH) and readily crosses the blood brain barrier [3,7,8]. It has been demonstrated that the 3α-hydroxysteroid is a potent anxiolytic, anticonvulsant, sedative/hypnotic and anesthetic, which exerts its effects through allosteric modulation of the gamma-aminobutyric acid (GABA) receptor complex. By binding to $GABA_A$ receptors, allopregnanolone (alloP) increases the binding of benzodiazepines and GABA to neuronal membranes resulting in increases in the influx of Cl^-. Thus, it has been suggested that alloP enhances GABA-mediated inhibition during states of hyperexcitability of the CNS, such as stress or anxiety [9-13].

Estrogens stimulate the hypothalamic-pituitary-adrenal axis (HPA). This can be observed by: 1) the presence of high levels of free cortisol, both in the morning and in the evening, by pregnant women or women receiving high doses of estrogens [14]; 2) the greater responsiveness of the HPA axis in women compared to men [14]; and 3) the acute administration of estradiol to healthy men in a psychosocial stress situation, which results in hyper responsiveness of the HPA axis and norepinephrine [15]. Furthermore, administration of estradiol to ovariectomized mice increases anxiety in situations of potential threat [16].

In addition to all these effects, estrogens and progesterone can change the density of $GABA_A$ receptors in certain brain regions [17-19] and, as a consequence, they may also alter the effects of certain drugs, such as benzodiazepines. Considering that women are naturally exposed to monthly fluctuation of these hormones, it is possible that their response to benzodiazepines also change over the ovarian cycle.

Studies in rodents have demonstrated changes in the sensitivity to the anxiolytic effect of benzodiazepines during the estrous cycle [20-22]. Moreover, short-term exposure (48-72 h) of female rats to high concentrations of alloP (10 mg/kg in sesame oil) results in increased expression of the α_4 subunit of the $GABA_A$ receptor, with subsequent behavioral and pharmacological changes of GABAergic function, represented by increased anxiety and insensitivity to benzodiazepine [23,24] whereas long-term exposure of female rats to pregnancy-induced high concentrations of alloP results in anxiolysis [25].

In women, the response to benzodiazepines also seems to vary along the menstrual cycle. According to a preliminary study by

*Corresponding author: Flavia Teixeira-Silva, Departamento de Fisiologia, Centro de Ciências Biológicas e da Saúde, Universidade Federal de Sergipe, Cidade Universitária 'Prof. José Aloísio de Campos', 49100-000, Sergipe, Brazil, Tel: +55-79-2105-6645; E-mail: teixeira_silva@terra.com.br

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Sundströn et al. [26], the sensitivity of healthy women to the sedative effect of diazepam, measured by saccadic eye movement velocity, is greater in the luteal phase compared to the follicular phase.

These observations acquire clinical importance when one considers that women suffer more of anxiety disorders [27-29], receiving, as a result, more benzodiazepine prescriptions [30,31].

Taking all these points into consideration, the aim of this study was to investigate the possible influence of the ovarian cycle in the acute effect of diazepam, in healthy women submitted to an experimental model of anxiety.

Materials and Methods

Participants

Fifty-eight undergraduate and graduate student volunteers, aged between 18 and 35, were selected for inclusion in the study using the following: a clinical structured questionnaire, a translated and adapted version of a Premenstrual Assessment Form [32]; a translated and adapted version of the State-Trait Anxiety inventory - Part II (STAI-trait) [33], a translated and adapted version of the Social Phobia Inventory (SPIN) [34]. Individuals possibly presenting pathologies that could interfere with the results or scoring above 19 on SPIN, women presenting premenstrual complaints and men scoring above 50 on STAI-trait (for comparison with a previous study) were excluded.

From the 58 volunteers, 40 women (20 in follicular phase and 20 in luteal phase) were selected for Experiment I, and 18 men for Experiment II.

This study was conducted with approval from the Ethics Committee in Research with Humans of the Universidade Federal de Sergipe, Brazil. Written informed consent was obtained from all participants.

Ovarian cycle phase determination

The women were randomly allocated to two different groups: 1) Follicular Phase; and 2) Luteal Phase. Subsequently, at least two of their ovarian cycles were monitored to check for length and regularity. Then, the experimental sessions were scheduled to coincide with one of the two ovarian phases as follows:

1. Follicular Phase: 6 to 10 days from the first day of the cycle (after menses);

2. Luteal Phase: 5 to 10 days after detection of urinary LH peak by an ovulation predictor kit (Baby Hopes®), which was used by the volunteers at home, after instructions.

Experimental model of anxiety

The Video-Monitored Stroop Color-Word Test, as standardized by Teixeira-Silva et al. [35], was used to elicit anxiety in the volunteers. In short, this test consists of presenting a board to the participant with one hundred of the color naming words blue, yellow, red, green and violet organized randomly in a 10 X 10 matrix. Each word is printed in a color different to its meaning, for example the word "red" printed in yellow ink. This board corresponds to the "Color-Word" card of the Stroop test [36]. To perform the task, the subject has to say, as quickly as possible and in the sequence presented, the names of the colors being seen (i.e. the color of the ink), but not the colors designated by the words. The task has to be performed in 2 minutes (maximum) and any errors are signaled with a bell. Skipping a color's sequence, hesitation in saying the color, and saying the color's "word" instead of its "ink" are

all considered to be errors. The whole test is videoed and presented to the subject on a monitor during the test.

Instructions were given to the subject using a CD recording which led them to believe that a group of professionals, located in another room, were observing them and would evaluate their performance.

Psychological measurements

State-Trait Anxiety Inventory (STAI) [33]: A validated Portuguese version was used [37].

Self-evaluation of tension level: This was performed using an analogical scale from 0 (totally relaxed) to 10 (extremely tense).

Visual Analogue Mood Scale (VAMS) [38]: This scale is composed of 16 pairs of opposite adjectives. Each pair is separated by a 100-mm horizontal line on which the subjects are requested to indicate with a vertical mark the point that best represents their feelings at that moment. The 16 items are distributed into four categories: (1) mental sedation (alert/drowsy, muzzy/clear headed, mentally slow/quick witted, attentive/dreamy); (2) physical sedation (strong/feeble, well-coordinated/clumsy, lethargic/energetic, incompetent/proficient); (3) tranquilization (calm/excited, contented/discontented, troubled/tranquil, tense/relaxed), and (4) other feelings and attitudes (happy/sad, antagonistic/amicable, interested/bored, withdrawn/gregarious). The range of values, for each category, is 0 – 400 mm. A Portuguese version, translated and validated by Zuardi and Karniol [39] and adapted by Del Porto et al. [40], was used.

Physiological measurements

Heart rate: Derived from two active Ag/AgCl electrodes placed on each side of the thorax, just above the nipple.

Electromyogram activity: Derived from two Ag/AgCl electrodes placed on the gastrocnemius muscle (part of the fight/flight response) at the nondominant leg.

1. The skin was cleaned with a mixture of alcohol/ether (90:10, V/V) before placement of the electrodes.

2. The recordings were made using a computerized system for monitoring physiological responses (I-330-C2+ Physiological Monitoring System, J&J Engineering, USA). All tests were performed in a quiet room maintained at a temperature between 22 and 25°C.

Drugs

Placebo: Corn Starch - 10 mg (Souza Farmácia de Manipulação).

Diazepam: Diempax - 10 mg (Sanofi Winthrop).

Procedure

Two experiments were performed. The first one aimed to investigate the possible influence of the ovarian cycle in the anxiolytic and hypnotic/sedative action of diazepam in volunteers submitted to the VMSCWT. The second one aimed simply to verify the validity of the experimental conditions in our laboratory.

In both experiments, the selected volunteers attended the laboratory on two consecutive days. The first day was used for adaptation, and the second for the actual test.

Adaptation day: The participants were taken to the experiment room, which was already organized and equipped with the necessary apparatus for the execution of the test. After filling in a consent form and then resting for about 5 min, the participants were submitted

to the psychological evaluations and then to 5 min of physiological recordings.

Test day: The participants were again taken to the experiment room, where they rested for about 5 min and were then submitted to the actual test, which underwent the same experimental design in both experiments.

Experimental design

In both experiments I and II, a capsule containing placebo (PBO), or 10 mg of diazepam (DZP) was administered to the volunteers. Before treatment, the adaptation procedure from the first day was repeated and the psychological and physiological data collected were labeled as the "Time 1" experimental situation. Following treatment, the volunteers were left in a comfortable waiting room for a period of 60 min, during which they could watch television or read magazines. Immediately after this time, before being given the test instructions, the participant had his/her psychological and physiological parameters evaluated. The psychological tests used were the STAI-state, self-evaluation of tension level, and VAMS while the physiological measurements made were heart rate and gastrocnemius electromyogram activity. These data were labeled as the "Time 2" experimental situation. After listening to the recorded instructions, the participant then performed the task, during which his/her physiological measurements were recorded. After 50 words, a pause was made for a third set of psychological evaluations. These new data were labeled as the "Time 3" experimental situation. Immediately following the evaluations, the test was restarted and continued up to the last color or until the end of the scheduled time. The participant then rested for 5 min, after which all the physiological and psychological parameters were again evaluated. This final set of data was labeled as the "Time 4" experimental situation.

Statistical analyses

The data collected during the adaptation phase were not analyzed as this situation was only intended to habituate the participants to the environment and apparatus that would be used on the following day.

For both experiments, the results collected on the test day were first analyzed using Kolmogorov–Smirnov's test for normal distribution and Bartlett's test for the homogeneity of variances. No impediments to the use of parametric tests were found for any of the evaluated parameters.

All the data obtained were analyzed using a two-factor analysis of variance (ANOVA) for repeated measures followed by Tukey's test for post hoc comparisons. For the cases in which the interaction between factors presented a significant difference, the analyses were followed by an ANOVA of simple main effect conducted on the situation variable for each group of volunteers.

Mauchly's test for sphericity (an assumption in within-participants ANOVA) was also performed on the repeated-measures factor of the ANOVA. Data which failed this test were subjected to the Huynh–Feldt correction which adjusts the degrees of freedom. An effect of this correction is that he degrees of freedom are subsequently expressed as decimals.

The STAI-trait and SPIN scores used in the selection process were analyzed by Student's t test. All significance tests were two tailed and were performed at the 5% significance level.

Results

To aid visualization of the results, the graphs shown here represent the profile of the responses presented by the volunteers, with the Time 2 being considered as the "zero" point for the changes induced by the test. As the analyses involved repeated measures of the same individuals, representation of the dispersion of the data provides little information and therefore error bars have been omitted from the graphs. Nevertheless, the means and standard deviations of the absolute values of the results for each of the psychological and physiological parameters are presented in Tables 1 and 2.

Experiment I

In this experiment, women in different ovarian cycle phases (follicular and luteal) were analyzed separately by two-way ANOVA, with treatment (PBO, DZP) and situation (Time 1, Time 2, Time 3, Time 4) as factors. There were 20 women for each cycle phase, 10 randomly allocated to the DZP group and 10 to the PBO group.

Psychological measurements

STAI-state: (Figure 1).

Follicular phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. The situation effect was significant [$F(2.31, 41.67) = 12.42$; $p < 0.000001$]. State-anxiety level was higher during Time 1 ($p \leq 0.001$), Time 3 ($p \leq 0.0001$), and Time 4 ($p \leq 0.02$), in relation to Time 2. The treatment effect was not significant.

Luteal phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. The situation effect was significant [$F(2.14, 38.51) = 21.33$; $p \leq 0.00005$]. State-anxiety level was higher during Time 3 ($p \leq 0.0001$), but not during Time 4, in relation to Time 2. Pre- and post-drug (Times 1 and 2) values did not differ significantly. The treatment effect was not significant.

Self-evaluation of tension level: (Figure 2).

Follicular phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. The situation effect was significant [$F(3, 54) = 18.96$; $p < 0.000001$]. Tension level was higher during Time 1 ($p \leq 0.001$) and Time 3 ($p \leq 0.0001$), but not during Time 4, in relation to Time 2. The treatment effect was not significant.

Luteal phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. The situation effect was significant [$F(2.36, 42.48) = 29.10$; $p < 0.000001$]. Tension level was higher during Time 1 ($p \leq 0.04$) and Time 3 ($p \leq 0.0001$), but not during Time 4, in relation to Time 2. The treatment effect was not significant.

VAMS—mental sedation: (Figure 3).

Follicular phase: The interaction effect between situation and treatment was significant [$F(3, 54) = 3.11$; $p \leq 0.03$]. Analysis of the situation as a single factor for the PBO group did not show any significant differences, although this analysis did reveal differences for the DZP group [$F(3, 27) = 4.60$; $p \leq 0.009$]. Self-reported mental sedation scores were greater during Time 2 ($p \leq 0.02$) in relation to Time 1.

Luteal phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. Neither the situation nor the treatment effects were significant.

VAMS-physical sedation: (Figure 4).

| Parameters	Ovarianphase	TT[a]	Situation[b]			
			Time	Time 2	Time 3	Time 4
STAI-state (points)	Follicular	PBO	32.5 ± 5.9	28.0 ± 5.0	36.4 ± 7.7	31.8 ± 6.1
		DZP	32.6 ± 3.1	29.3 ± 3.5	36.7 ± 6.5	31.5 ± 3.5
	Luteal	PBO	33.8 ± 5.5	32.5 ± 5.0	38.1 ± 5.4	35.5 ± 6.2
		DZP	36.6 ± 11.0	32.6 ± 6.7	41.0 ± 5.8	35.4 ± 5.7
Tension level (points)	Follicular	PBO	2.9 ± 2.6	1.5 ± 1.5	4.5 ± 2.3	2.4 ± 2.5
		DZP	2.2 ± 1.5	1.1 ± 0.9	2.7 ± 1.8	1.6 ± 1.5
	Luteal	PBO	2.0 ± 0.9	1.3 ± 1.1	3.5 ± 2.0	2.0 ± 1.2
		DZP	2.0 ± 2.2	0.9 ± 1.0	4.6 ± 1.7	1.9 ± 1.7
Mental sedation (mm)	Follicular	PBO	93.1 ± 69.3	93.0 ± 82.0	117.7 ± 89.7	80.6 ± 78.9
		DZP	85.4 ± 58.7	173.5±101.2	175.3±101.2	161.5±100.5
	Luteal	PBO	84.6 ± 42.0	109.6 ± 66.6	119.6 ± 83.6	117.5 ± 93.8
		DZP	141.8 ± 90.3	172.9±106.6	159.3±75.3	169.1±110.3
Physical sedation (mm)	Follicular	PBO	64.4 ± 56.8	64.1 ± 66.7	79.8 ± 70.6	64.5 ± 61.7
		DZP	76.8 ± 56.9	142.3 ± 89.7	152.0 ± 84.2	122.4 ± 80.6
	Luteal	PBO	72.8 ± 36.6	75.0 ± 52.2	97.9 ± 58.8	93.7 ± 63.4
		DZP	136.7 ± 87.5	145.7 ± 98.7	156.4 ± 65.1	147.6 ± 93.2
Tranquilization (mm)	Follicular	PBO	326.8 ± 46.7	353.2 ± 62.1	282.9 ± 95.0	333.4 ± 57.9
		DZP	317.1 ± 50.3	342.8 ± 49.5	285.6 ± 63.4	331.8 ± 47.4
	Luteal	PBO	312.9 ± 66.0	345.0 ± 40.3	279.2 ± 81.8	313.7 ± 57.8
		DZP	265.9 ±102.8	315.8 ± 36.8	252.9±86.0	296.4 ± 30.9
Others Feelings and Attitudes (mm)	Follicular	PBO	52.0 ± 50.1	46.6 ± 55.2	50.5 ± 51.6	46.8 ± 54.9
		DZP	65.5 ± 57.3	65.2 ± 53.0	66.3 ± 50.0	67.2 ± 49.9
	Luteal	PBO	73.8 ± 48.8	72.8 ± 56.4	71.3 ± 45.9	73.7 ± 47.7
		DZP	102.0 ± 62.0	100.4 ± 39.8	101.7 ± 44.8	112.0 ± 54.7
Heart rate (beats/min)	Follicular	PBO	79.9 ± 7.5	69.6 ± 6.3	94.7 ± 14.7	72.0 ± 7.5
		DZP	81.7 ± 7.3	74.7 ± 9.3	88.7 ± 10.5	75.3 ± 7.4
	Luteal	PBO	82.5 ± 14.0	76.7 ± 10.1	95.6 ± 15.1	79.0 ± 10.9
		DZP	80.6 ± 8.0	73.0 ± 10.9	95.2 ± 16.0	73.6 ± 10.6
EMG – Gastrocnemius (µV)	Follicular	PBO	0.9 ± 0.5	1.1 ± 0.5	3.1 ± 2.1	1.4 ± 0.6
		DZP	1.5 ± 1.4	1.1 ± 0.8	2.8 ± 2.4	0.9 ± 0.8
	Luteal	PBO	1.8 ± 2.5	1.1 ± 0.8	1.8 ± 1.0	0.8 ± 0.4
		DZP	0.8 ± 0.3	0.9 ± 0.5	2.1 ± 1.2	0.9 ± 0.5

Note: [a]TT: Treatment, [b]Data are presented as mean ± S.D.

Table 1: Summary of the absolute values obtained in Experiment I.

| Parameters	TT[a]	Situation[b]			
		Time 1	Time 2	Time 3	Time 4
STAI-state (points)	PBO	36.8 ± 4.0	34.9 ± 4.5	42.2 ± 7.8	38.8 ± 6.7
	DZP	34.1 ± 10.7	31.0 ± 8.3	34.2 ± 10.1	31.4 ± 9.1
Tension level (points)	PBO	2.1 ± 1.2	1.2 ± 1.4	4.4 ± 2.6	1.8 ± 1.3
	DZP	2.6 ± 1.0	1.1 ± 1.1	2.8 ± 2.0	1.3 ± 1.2
Mental sedation (mm)	PBO	113.6 ± 83.5	119.3 ± 73.2	136.4 ± 61.6	103.1 ± 53.9
	DZP	112.4 ± 75.0	154.7 ± 87.6	172.2 ± 89.9	130.4 ± 78.4
Physical sedation (mm)	PBO	128.9 ± 64.1	130.4 ± 53.2	133.1 ± 38.4	127.3 ± 53.8
	DZP	85.4 ± 63.1	112.6 ± 77.6	120.6 ± 60.9	97.9 ± 72.2
Tranquilization (mm)	PBO	279.0 ± 80.3	311.6 ± 62.0	246.0 ± 89.9	268.4 ± 71.2
	DZP	312.1 ± 64.8	322.2 ± 64.5	266.0 ± 56.6	310.6 ± 60.6
Others Feelings and Attitudes (mm)	PBO	76.7 ± 48.6	84.8 ± 57.8	98.6 ± 62.7	91.3 ± 57.4
	DZP	70.2 ± 52.7	71.4 ± 58.2	78.8 ± 59.7	68.4 ± 50.1
Heart rate (beats/min)	PBO	77.3 ± 18.0	77.7 ± 17.3	94.7 ± 14.7	68.2 ± 15.8
	DZP	79.2 ± 5.1	72.4 ± 7.8	92.2 ± 17.1	73.6 ± 6.2
EMG – Gastrocnemius (µV)	PBO	0.8 ± 0.3	0.9 ± 0.4	2.7 ± 2.3	1.1 ± 0.5
	DZP	1.7 ± 2.9	1.5 ± 1.7	4.5 ± 5.1	1.3 ± 0.8

Note: [a]TT: Treatment, [b]Data are presented as mean ± S.D.

Table 2: Summary of the absolute values obtained in Experiment II.
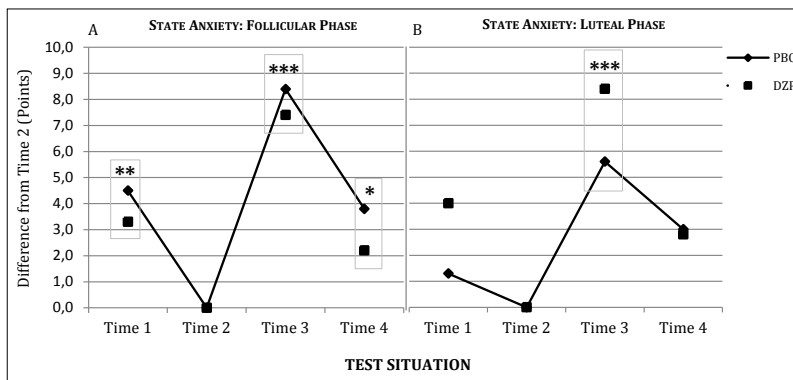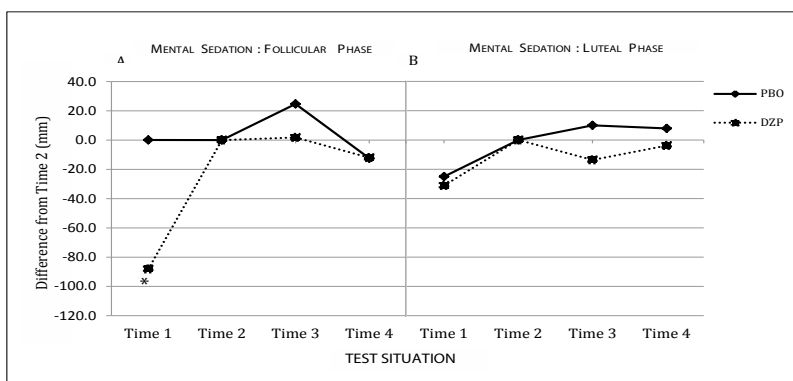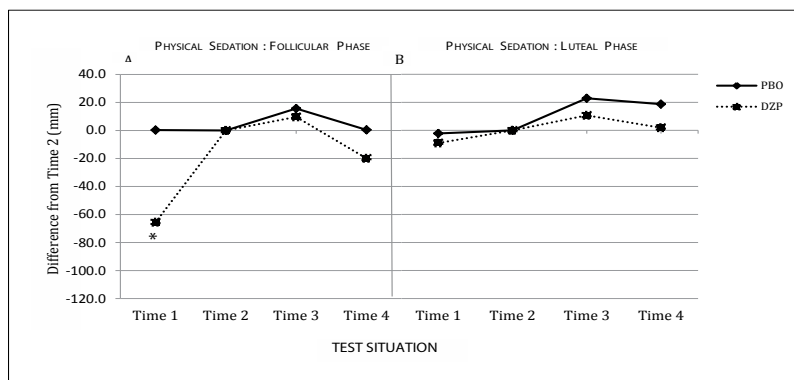
Note: A: Women in follicular phase. B: Women in luteal phase. Time 1: pre-drug; Time 2: post-drug, before the test; Time 3: during the test; Time 4: after the test. *Significantly different from Time 2 (p ≤ 0.05). **Significantly different from Time 2 (p ≤ 0.005). ***Significantly different from Time 2 (p ≤ 0.0005).

Figure 1: State anxiety levels of women, treated with diazepam (DZP) or placebo (PBO), in response to the anxiogenic test.
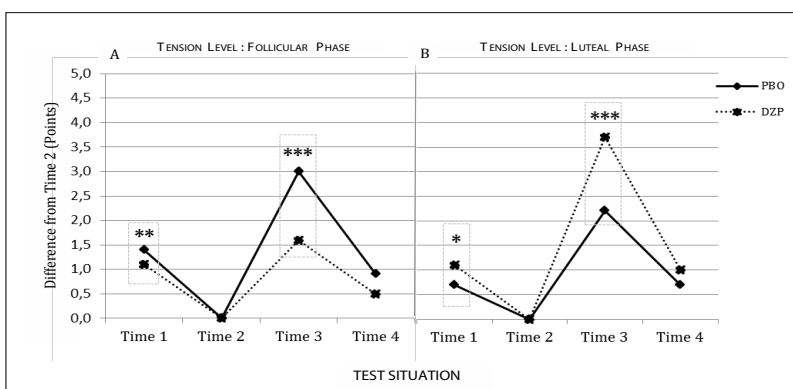
Note: A: Women in follicular phase. B: Women in luteal phase. Time 1: pre-drug; Time 2: post-drug, before the test; Time 3: during the test; Time 4: after the test. *Significantly different from Time 2 (p ≤ 0.05). **Significantly different from Time 2 (p ≤ 0.005). ***Significantly different from Time 2 (p ≤ 0.0005).

Figure 2: Subjective tension levels of women, treated with diazepam (DZP) or placebo (PBO), in response to the anxiogenic test.

Note: A: Women in follicular phase. B: Women in luteal phase. Time 1: pre-drug; Time 2: post-drug, before the test; Time 3: during the test; Time 4: after the test. *Significantly different from Time 2 (p ≤ 0.05).

Figure 3: Interaction between test situation and treatment for mental sedation levels of women, treated with diazepam (DZP) or placebo (PBO), in response to the anxiogenic test.

Note: A: Women in follicular phase. B: Women in luteal phase. Time 1: pre-drug; Time 2: post-drug, before the test; Time 3: during the test; Time 4: after the test. *Significantly different from Time 2 (p ≤ 0.05).

Figure 4: Interaction between test situation and treatment for physical sedation levels of women, treated with diazepam (DZP) or placebo (PBO), in response to the anxiogenic test.

Follicular phase: The interaction effect between situation and treatment was significant [F(2.17, 39.09) = 3.18; p ≤ 0.05]. Analysis of the situation as a single factor for the PBO group did not show any significant differences, although this analysis did reveal differences for the DZP group [F(2.20, 38) = 4.40; p ≤ 0.02], as the mental sedation scores were greater during Time 2 (p ≤ 0.02) in relation to Time 1.

Luteal phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. Neither the situation nor the treatment effects were significant.

VAMS—tranquilization

Follicular phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. The situation effect was significant [F(2.57, 46.33) =12.53; p ≤ 0.00001]. Tranquilization scores were smaller during Time 3 (p ≤ 0.0001), but not during Time 4, in relation to Time 2. Pre- and post-drug (Times 1 and 2) values did not differ significantly. The treatment effect was not significant.

Luteal phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. The situation effect was significant [F(2.20, 39.50) = 5.07; p ≤ 0.01]. Tranquilization scores were smaller during Time 3 (p ≤ 0.002), but not during Time 4, in relation to Time 2. Pre- and post-drug (Times 1 and 2) values did not differ significantly. The treatment effect was not significant.

VAMS—other feelings and attitudes

Follicular phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. Neither the situation nor the treatment effects were significant.

Luteal phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. Neither the situation nor the treatment effects were significant.

Physiological measurements

Heart rate

Follicular phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. The situation effect was significant [F(1.60, 25.67) = 33.52; p < 0.000001]. Heart rate was higher during Time 1 (p ≤ 0.0007) and

Time 3 (p ≤ 0.0001), but not during Time 4, in relation to Time 2. The treatment effect was not significant.

Luteal phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. The situation effect was significant [F(1.37, 24.64) = 26.29; p ≤ 0.000006]. Heart rate was higher during Time 1 (p ≤ 0.05) and Time 3 (p ≤ 0.0001), but not during Time 4, in relation to Time 2. The treatment effect was not significant.

Gastrocnemius electromyogram

Follicular phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. The situation effect was significant [F(1.31, 23.66) = 10.17; p ≤ 0.002]. Muscular tension was higher during Time 3 (p ≤ 0.0002), but not during Time 4, in relation to Time 2. Pre- and post-drug (Times 1 and 2) values did not differ significantly. The treatment effect was not significant.

Luteal phase: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. Neither the situation nor the treatment effects were significant.

STAI-trait

Follicular phase: The level of trait anxiety was not significantly different between the groups PBO (mean ± S.D. = 40.0 ± 9.1) and DZP (mean ± S.D. = 40.2 ± 9.4).

Luteal phase: The level of trait anxiety was not significantly different between the groups PBO (mean ± S.D. = 44.7 ± 10.7) and DZP (mean ± S.D. = 41.9 ± 8.6).

SPIN

Follicular phase: The scores of social phobia were not significantly different between the groups PBO (mean ± S.D. = 13.6 ± 4.7) and DZP (mean ± S.D. = 10.8 ± 5.9).

Luteal phase: The scores of social phobia were not significantly different between the groups PBO (mean ± S.D. = 12.9 ± 5.2) and DZP (mean ± S.D. = 11.4 ± 5.7).

Experiment II

In this experiment, men were analyzed using a two-way ANOVA, with treatment (PBO and DZP) and situation (Time 1, Time 2, Time 3 and Time 4) as factors. There were nine volunteers per group.

Psychological measurements

STAI-state: (Figure 5).

The interaction effect between situation and treatment was significant [$F(3, 48) = 3.16$; $p = 0.03$]. Analysis of the situation as a single factor for the PBO group revealed significant differences [$F(3, 24) = 9.29$; $p \leq 0.0002$], as the anxiety level was higher during Time 3 ($p \leq 0.0003$), but not during Time 4, in relation to Time 2. Pre- and post-drug (Times 1 and 2) values did not differ significantly. The same analyses for the DZP group did not reveal any significant differences.

Self-evaluation of tension level: (Figure 6).

The interaction effect between situation and treatment was significant [$F(3, 48) = 2.97$; $p \leq 0.04$]. Analysis of the situation as a single factor for the PBO group revealed significant differences [$F(3, 24) = 13.51$; $p \leq 0.00002$], as the anxiety level was higher during Time 3 ($p \leq 0.0001$), but not during Time 4, in relation to Time 2. Pre- and post-drug (Times 1 and 2) values did not differ significantly. The DZP group also presented significant differences [$F (1.88, 15.10) = 5.96$, $p \leq 0.01$], as the anxiety level was higher during Time 1 ($p \leq 0.03$) and Time 3 ($p \leq 0.01$), in relation to Time 2, but these differences were less pronounced than for the PBO group.
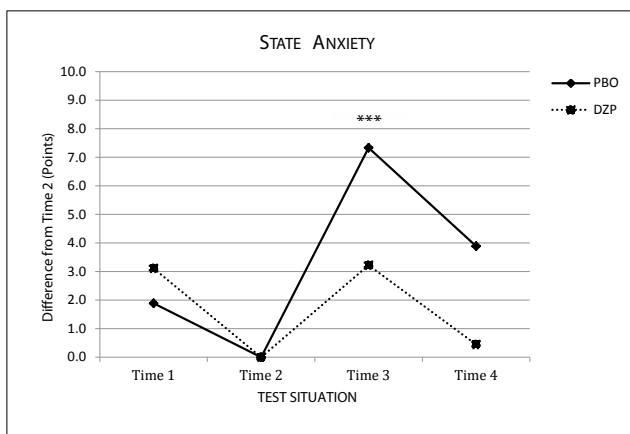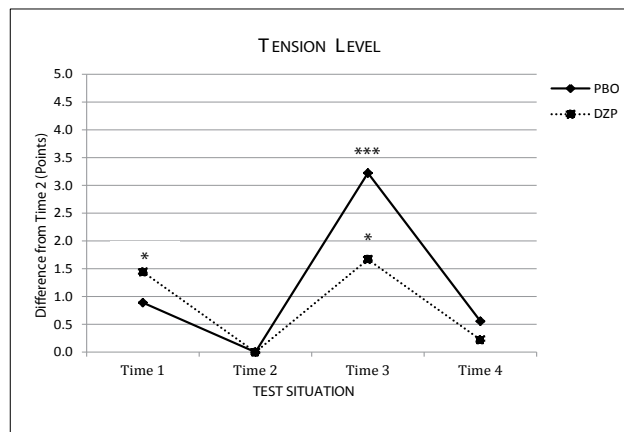
VAMS—mental sedation: (Figure 7).

The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. Neither the situation nor the treatment effects were significant.

VAMS—physical sedation: (Figure 8).

The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. Neither the situation nor the treatment effects were significant.

VAMS—tranquilization: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. The situation effect was significant [$F(3, 48) = 11.87$; $p \leq 0.000006$]. Tranquilization scores were smaller during Time 3 ($p \leq 0.0001$), but not during Time 4, in relation to Time 2. Pre- and post-drug (Times 1 and 2) values did not differ significantly. The treatment effect was not significant.
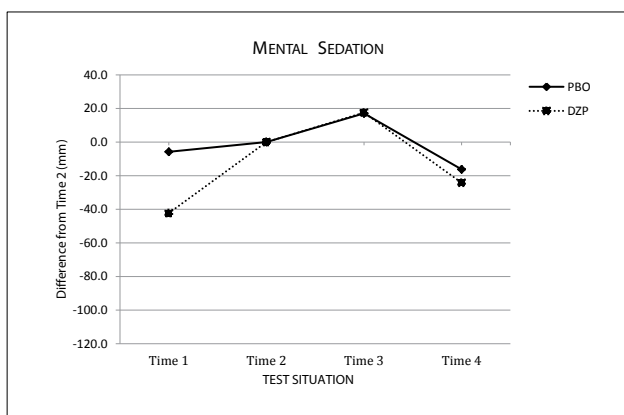

Note: Time 1: pre-drug; Time 2: post-drug, before the test; Time 3: during the test; Time 4: after the test. ***Significantly different from Time 2 ($p \leq 0.0005$).

Figure 6: Interaction between test situation and treatment for tension levels of men, treated with diazepam (DZP) or placebo (PBO), in response to the anxiogenic test.

Note: Time 1: pre-drug; Time 2: post-drug, before the test; Time 3: during the test; Time 4: after the test.

Figure 7: Mental sedation levels of men, treated with diazepam (DZP) or placebo (PBO), in response to the anxiogenic test.

Note: Time 1: pre-drug; Time 2: post-drug, before the test; Time 3: during the test; Time 4: after the test.

Figure 8: Physical sedation levels of men, treated with diazepam (DZP) or placebo (PBO), in response to the anxiogenic test.

Note: Time 1: pre-drug; Time 2: post-drug, before the test; Time 3: during the test; Time 4: after the test. ***Significantly different from Time 2 ($p \leq 0.0005$).

Figure 5: Interaction between test situation and treatment for state-anxiety levels of men, treated with diazepam (DZP) or placebo (PBO), in response to the anxiogenic test.
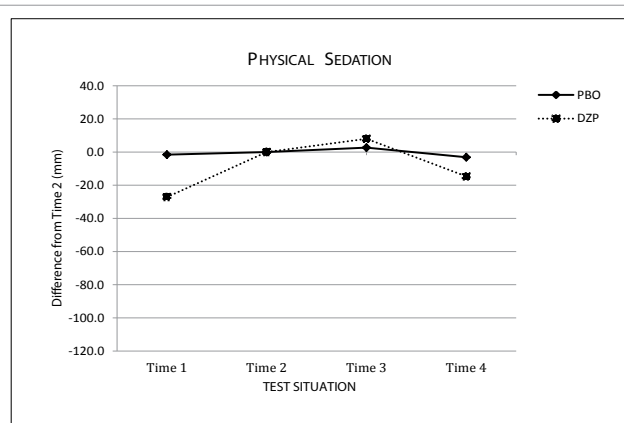
VAMS—other feelings and attitudes: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. Neither the situation nor the treatment effects were significant.

Physiological measurements

Heart rate: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. The situation effect was significant [$F_{(2.66, 42.60)}$ =27.41; p <0.000001]. Heart rate was higher during Time 3 ($p \leq 0.0001$), but not during Time 4, in relation to Time 2. Pre- and post-drug (Times 1 and 2) values did not differ significantly. The treatment effect was not significant.

Gastrocnemius electromyogram: The interaction effect between situation and treatment was not significant and so the two main effects were analyzed. The situation effect was significant [$F_{(1.43, 22.84)}$ = 5.85; $p \leq 0.01$]. Muscular tension was higher during Time 3 ($p \leq 0.005$), but not during Time 4, in relation to before. Pre- and post-drug (Times 1 and 2) values did not differ significantly. The treatment effect was not significant.

STAI-trait

The level of trait anxiety was not significantly different between the groups PBO (mean ± S.D. = 38.6 ± 3.9) and DZP (mean ± S.D. = 37.4 ± 9.5).

SPIN

The scores of social phobia were not significantly different between the groups PBO (mean ± S.D. = 10.7 ± 6.9) and DZP (mean ± S.D. = 9.5 ± 5.4).

Discussion

The aim of this work was to investigate the possible influence of the ovarian cycle in the classical effects of diazepam in healthy women submitted to an experimental model of anxiety.

The results of Experiment I showed that women in the follicular ovarian phase presented no response to the anxiolytic action of diazepam, since the increase in anxiety promoted by the anxiogenic VMSCWT was neither prevented nor reduced by the drug, as demonstrated by the STAI-state scores, the self-evaluated tension levels and the values of the tranquilization factor of the VAMS. In contrast, these women did show an increase in their levels of mental and physical sedation in response to diazepam. These results replicate the findings of Teixeira-Silva et al. [35].

Surprisingly, diazepam also did not present an anxiolytic effect in women in the luteal ovarian phase neither presented a hypnotic/sedative action. These intriguing results show a complete lack of response to the effects of the acute administration of diazepam by women in the luteal phase. In order to verify if this finding was not due to flaws in the execution of the methods or in the drug used, Experiment II was conducted, only with men. Now, the diazepam action profile was exactly what expected, taking into consideration a previous study [35]. The drug prevented the increase in anxiety during the test, as demonstrated by the STAI-state and by the self-evaluation of tension level, without causing sedation. Therefore, the results obtained with women cannot be attributed to failure in the experimental procedure.

So what could explain the different response profiles to acute diazepam administration between men and women and between women in different phases of the ovarian cycle?

Possible pharmacokinetic differences could account for all different response patterns observed here. However, it has been demonstrated that there is no difference in diazepam bioavailability between men and women, when orally administered [41], while data about benzodiazepine pharmacokinetic changes across the ovarian cycle are scarce. To the best of our knowledge, there are no studies concerning the influence of the menstrual cycle on diazepam pharmacokinetics. Anyhow, it has been demonstrated that the pharmacokinetics of orally administered alprazolam does not change through the ovarian cycle [42]. The same has been observed for the intravenous administration of midazolam [43].

Having said all that, it is not possible to rely on pharmacokinetics to explain our results. Thus, pharmacodynamic differences may be responsible for these findings.

What would make women in the follicular phase, unlike men, irresponsive to the anxiolytic action of diazepam?

This would suggest a decreased sensitivity to diazepam of women in the follicular phase in comparison to men. In fact, it has been shown that diazepam has an anxiolytic effect in men in a lower dose (5 mg), which did not have any effect at all in women in the follicular phase [35]. A possible explanation of this could be the low levels of progesterone and it metabolites in this phase, especially alloP, which potentiates the action of benzodiazepines [44]. However, with 10 mg of diazepam in the present study, as in a previous one [35], these women already felt the hypnotic/sedative effect of the drug, which is not seen in men. Therefore, there was a difference in type of response and not just a dose-response curve dislocation.

Fluctuations of the sex steroid levels during the ovarian cycle are strongly associated with changes in the of the $GABA_A$ receptor subunits composition in the brain. Evidence in rodents, monkeys and cell models show that estrogens, progesterone and alloP influence the expression pattern of $GABA_A$ receptor subunits [45-51]. Variable subunits expression patterns define the $GABA_A$/BZD receptor subtype. Those containing $\alpha_1\beta\gamma_2$ subunits correspond to BZ1 receptor, which mediates the sedative effect of diazepam, while those containing α_2, α_3 or α_5 in combination with the β and γ_2 subunits correspond to BZ2 receptor, which is responsible for the anxiolytic effect [52-54]. Considering this, although there are no studies to support this idea, it is tempting to speculate that women in the follicular phase present more BZ1 and less BZ2 receptors. Future research could investigate this further. On the other hand, neither the anxiolytic nor the hypnotic/sedative effects were present in women in the luteal phase.

What could be the factor responsible for abolishing both anxiolytic and hypnotic/sedative effects of diazepam in women in the luteal phase?

In studies by Gulinello et al. [23,24], it was found that short-term exposure (48-72 h) to high concentrations of alloP to female rats led to a lack of lorazepam's anxiolytic effect, while it increased the α_4 subunit expression of the $GABA_A$ receptor, which is insensitive to the action of benzodiazepines. However, after long-term exposure (five days) to the neurosteroid, the α_4 subunit levels and the pharmacology of lorazepam returned to control values and remain unchanged until removal of the neurosteroid. Therefore, it is possible that exposure to alloP in women during the luteal phase corresponds to the short-term exposure in rats from Gulinello's studies.

In contradiction to this hypothesis, the preliminary study by Sundström et al. [26] found that responsiveness to diazepam in healthy women, assessed by the saccadic eye movement velocity, as a monitor

of sedation, was greater in the luteal phase than in the follicular phase. However, the period investigated by Sündstron et al. was the late luteal phase (1-7 days before the onset of menses), which could correspond to long-term exposure of the studies by Gulinello or a period of sex hormones decline.

At this point, it is also valid to consider the existence of differences between male and female brains, independently of the ovarian cycle, which could explain the lack of anxiolytic effect found, here, in both menstrual cycle phases. For example, a study by Ravenelle et al. [55], performed with rats, showed sex-differences in response to the anti-anxiety effects of diazepam that correlated with GABA neuron variations along stress regions. Males present a greater number of GABAergic interneurons in the amygdala, which could explain their greater response to diazepam.

Independently of its cause, which should be investigated in the future, the fact is that diazepam seems to be devoid of acute anxiolytic effect in women at any ovarian cycle phase. These findings, while intriguing, are not exactly unexpected. In another placebo-controlled study, where the anxiety was induced by a real-life stress situation (cholecystectomy) in women, the oral acute administration of diazepam (10 mg) was also unable to decrease anxiety [56].

In relation to the physiological parameters, the results showed that diazepam did not prevent the changes in heart rate and gastrocnemius tension, elicited by VMSCWT, neither in women nor men. Nevertheless, this inability of benzodiazepines to prevent or reduce anxiety-accompanied physiological alterations was already expected, as it has been shown in a variety of studies of real-life or laboratory-induced stress [35,57-59].

Taking all these points into consideration, the data presented here not only contributes for the understanding of the important relationship among anxiety, ovarian hormones and benzodiazepines, but also indicates that care must be exercised when prescribing benzodiazepines to women, at least for acute use, which is also common. In fact, one of the indications for the use of diazepam (10 mg) is for sedation and relief of anxiety, tension and stress prior to diagnostic or therapeutic procedures, as pointed out by the Valium® package insert itself. The acute administration of 5 to 10 mg of diazepam by oral route, usually one hour before the start of an invasive or unpleasant medical procedure is widely used as premedication or as the main pharmacological method for sedation in outpatient dental procedures [60-64].

The fact that diazepam is being prescribed to women, although it may not have the intended effect, is the reflection of the negligence in women biological research, which has negatively affected the understanding of women's physiology [65].

A survey of studies published in nine influential medical journals, in 2004, found that from 46 clinical trials, only 37% of participants were women and this proportion declined to 24% when the analysis was restricted to drug testing. In 87% of the studies, results were not reported by gender nor were gender included as a covariate [66].

Even though women seek treatment and receive prescription of psychotropic medications more than men, the majority of phase I clinical trials, which determine the therapeutic doses, are performed only with men [67-69].

These facts make research such as the present study crucial, and the knowledge derived from them should be transferred into clinical practice and become a key consideration in treatment choice by health professionals.

Conclusion

In summary, despite the fact that future investigation should be performed with long-term exposure to diazepam and with different benzodiazepines, the results presented here:

1. Show that the ovarian cycle does influence the effects of a single dose of diazepam;

2. Draw attention to the importance of considering the gender and also the ovarian cycle phase when studying the effects of drugs;

3. Indicate that, contrary to expectations, a single dose of diazepam is not a good prescription choice for reducing or preventing acute anxiety in women.

References

1. Baulieu EE, Robel P (1990) Neurosteroids: a new brain function? J Steroid Biochem Mol Biol 37: 395-403.

2. Ganong WF (2000) As gônadas: desenvolvimento e função do sistema reprodutor. In: Ganong WF. (ed) Fisiologia médica, Rio de Janeiro: McGraw-Hill Interamericana do Brasil 299-328.

3. Majewska MD (1987) Steroids and brain activity. Essential dialogue between body and mind. Biochem Pharmacol 36: 3781-3788.

4. Magiakou MA, Mastorakos G, Webster E, Chrousos GP (1997) The hypothalamic-pituitary-adrenal axis and the female reproductive system. Ann NY Acad Sci 816: 42-56.

5. Ter Horst GJ, Wichmann R, Gerrits M, Westenbroek C, Lin Y (2009) Sex differences in stress responses: focus on ovarian hormones. Physiol Behav 97: 239-249.

6. Lovick TA (2012) Estrous cycle and stress: influence of progesterone on the female brain. Braz J Med Biol Res 45: 314-320.

7. Ichikawa S, Sawada T, Nakamura Y, Morioka H (1974) Ovarian secretion of pregnane compounds during the estrous cycle and pregnancy in rats. Endocrinology 94: 1615-1620.

8. Compagnone NA, Mellon SH (2000) Neurosteroids: biosynthesis and function of these novel neuromodulators. Front Neuroendocrinol 21: 1-56.

9. Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM (1986) Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232: 1004-1007.

10. Harrison NL, Majewska MD, Harrington JW, Barker JL (1987) Structure-activity relationships for steroid interaction with the gamma-aminobutyric acid A receptor complex. J Pharmacol Exp Ther 241: 346-353.

11. Peters JA, Kirkness EF, Callachan H, Lambert JJ, Turner AJ (1988) Modulation of the GABAA receptor by depressant barbiturates and pregnane steroids. Br J Pharmacol 94: 1257-1269.

12. Bitran D, Hilvers RJ, Kellogg CK (1991) Anxiolytic effects of 3 alpha-hydroxy-5 alpha[beta]-pregnan-20-one: endogenous metabolites of progesterone that are active at the GABAA receptor. Brain Res 1991; 56: 157-61.

13. Purdy RH, Morrow AL, Moore PH Jr, Paul SM (1991) Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. Proc Natl Acad Sci USA 88: 4553-4557.

14. Chrousos GP, Torpy D, Gold PW (1998) Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. Ann Intern Med 129: 229-240.

15. Kirchbaum C, Schommer N, Federenko I, Gaab J, Neumann O, et al. (1996) Short-term estradiol treatment enhances pituitary adrenal axis and sympathetic responses to psychosocial stress in healthy young men. J Clin Endocrinol Metab 81: 3639-3643.

16. Morgan MA, Pfaff DW (2002) Estrogen's effects on activity, anxiety, and fear in two mouse strains. Behav Brain Res 132: 85-93.

17. Hamon M, Goetz C, Euvrard C, Pasqualini C, Le Dafniet M, et al. (1983) Biochemical and functional alterations of central GABA receptors during chronic estradiol treatment. Brain Res 279: 141-152.

18. Maggi A, Perez J (1984) Progesterone and estrogens in rat brain: modulation of GABA (gamma-aminobutyric acid) receptor activity. Eur J Pharmacol 103: 165-168.

19. Schumacher M, Coirini H, McEwen BS (1989) Regulation of high-affinity GABAa receptors in specific brain regions by ovarian hormones. Neuroendocrinology 50: 315-320.

20. Fernández-Guasti A, Picazo O (1990) The actions of diazepam and serotonergic anxiolytics vary according to the gender and the estrous cycle phase. Pharmacol Biochem Behav 37: 77-81.

21. Carey MP, Billing AE, Fry JP (1992) Fluctuations in responses to diazepam during the oestrous cycle in the mouse. Pharmacol Biochem Behav 41: 719-725.

22. Bitran D, Dowd JA (1996) Ovarian steroids modify the behavioral and neurochemical responses of the central benzodiazepine receptor. Psychopharmacology 125: 65-73.

23. Gulinello M, Gong QH, Li X, Smith SS (2001) Short-term exposure to a neuroactive steroid increases a4 GABAA receptor subunit levels in association with increased anxiety in the female rat. Brain Res 910: 55-66.

24. Gulinello M, Smith SS (2003) Anxiogenic effects of neurosteroid exposure: sex differences and altered GABAA receptor pharmacology in adult rats. J Pharmacol Exp Ther 305: 541-548.

25. de Brito Faturi C, Teixeira-Silva F, Leite JR (2006) The anxiolytic effect of pregnancy in rats is reversed by finasteride. Pharmacol Biochem Behav 85: 569-574.

26. Sundström I, Ashbrook D, Bäckström T (1997) Reduced benzodiazepine sensitivity in patients with premenstrual syndrome: a pilot study. Psychoneuroendocrinology 22: 25-38.

27. Regier DA, Boyd JH, Burke JD Jr, Rae DS, Myers JK, et al. (1988) One-month prevalence of mental disorders in the United States. Based on five Epidemiologic Catchment Area sites. Arch Gen Psychiatry 45: 977-986.

28. Muranetti CL, Terra MB (2007) Transtornos de ansiedade: um estudo de prevalência e comorbidade com o tabagismo em um ambulatório de psiquiatria. J Bras Psiquiatr 2007; 56(2): 108-115.

29. Lebron-Milad K, Milad MR (2012) Sex differences, gonadal hormones and the fear extinction network: implications for anxiety disorders. Biol Mood Anxiety Disord 2: 3.

30. Beary MD, Lacey JH, Bhat AV (1983) The neuro-endocrine impact of 3-hydroxy-diazepam (temazepam) in women. Psychopharmacology (Berl) 79: 295-297.

31. Nordon DG, Akamine K, Novo NF, Hübner CK (2009) Características de uso de benzodiazepínicos por mulheres que buscavam tratamento na atenção primária. Rev Psiquiatr Rio Gd Sul 31: 3.

32. Pires MLN (1996) Disforia Pré-Menstrual: evolução do conceito, dificuldades diagnósticas e aspectos terapêuticos - efeitos da privação parcial de sono. São Paulo.

33. Spielberger CD, Gorsuch RL, Lushene RE (1970) Manual for the state–trait anxiety inventory ("self-evaluation questionnaire"). Palo Alto, CA: California Consulting-Psychologists Press.

34. Connor KM, Davidson JR, Churchill LE, Sherwood A, Foa E, et al. (2000) Psychometric properties of the Social Phobia Inventory (SPIN). New self-rating scale. Br J Psychiatry 176: 379-386.

35. Teixeira-Silva F, Prado GB, Ribeiro LC, Leite JR (2004) The anxiogenic video-recorded Stroop Color-Word Test: psychological and physiological alterations and effects of diazepam. Physiol Behav 82: 215-230.

36. Stroop JR (1935) Studies of interference in serial verbal reactions. J Exp Psychol 18: 643-662.

37. Biaggio AMB, Natalício L (1979) Manual para o Inventário de Ansiedade Traço-Estado (IDATE). Rio de Janeiro: Centro Editor de Psicologia Aplicada - CEPA.

38. Norris H (1971) The action of sedatives on brain stem oculomotor systems in man. Neuropharmacology 10: 181-191.

39. Zuardi AW, Karniol IG (1981) Estudo transcultural de uma escala de auto-avaliação para estudos subjetivos. J Bras Psiquiatr 30: 403-406.

40. Del Porto JA, Laranjeiras RR, Masur J (1983) Escalas de auto-avaliação de estados subjetivos—influência das instruções. J Bras Psiquiatr 32: 87-90.

41. Divoll M, Greenblatt DJ, Ochs HR, Shader RI (1983) Absolute bioavailability of oral and intramuscular diazepam: effects of age and sex. Anesth Analg 62: 1-8.

42. Kirkwood C, Moore A, Hayes P, DeVane CL, Pelonero A (1991) Influence of menstrual cycle and gender on alprazolam pharmacokinetics. Clin Pharmacol Ther 50: 404-409.

43. Kharasch ED, Mautz D, Senn T, Lentz G, Cox K (1999) Menstrual cycle variability in midazolam pharmacokinetics. J Clin Pharmacol 39: 275-280.

44. Brot MD, Akwa Y, Purdy RH, Koob GF, Britton KT (1997) The anxiolytic-like effects of the neurosteroid allopregnanolone: interactions with GABA(A) receptors. Eur J Pharmacol 325: 1-7.

45. Yu R, Follesa P, Ticku MK (1996) Down-regulation of the GABA receptor subunits mRNA levels in mammalian cultured cortical neurons following chronic neurosteroid treatment. Brain Res Mol Brain Res 41: 163-168.

46. Herbison AE, Fénelon VS (1995) Estrogen regulation of GABAA receptor subunit mRNA expression in preoptic area and bed nucleus of the stria terminalis of female rat brain. J Neurosci 15: 2328-2337.

47. Weiland NG, Orchinik M (1995) Specific subunit mRNAs of the GABAA receptor are regulated by progesterone in subfields of the hippocampus. Brain Res Mol Brain Res 32: 271-278.

48. Follesa P, Serra M, Cagetti E, Pisu MG, Porta S, et al. (2000) AlloP synthesis in cerebellar granule cells: Roles in regulation of GABAA receptor expression and function during progesterone treatment and withdrawal. Mol Pharmacol 2000; 57: 1262-1270.

49. Jorge JC, McIntyre KL, Henderson LP (2002) The function and the expression of forebrain GABA(A) receptors change with hormonal state in the adult mouse. J Neurobiol 50: 137-149.

50. Lovick TA, Griffiths JL, Dunn SM, Martin IL (2005) Changes in GABA(A) receptor subunit expression in the midbrain during the oestrous cycle in Wistar rats. Neuroscience 131: 397-405.

51. Noriega NC, Eghlidi DH, Garyfallou VT, Kohama SG, Kryger SG, et al. (2010) Influence of 17-beta-estradiol and progesterone on GABAergic gene expression in the arcuate nucleus, amygdala and hippocampus of the rhesus macaque. Brain Res 1307: 28-42.

52. Whiting PJ (2003) GABA-A receptor subtypes in the brain: a paradigm for CNS drug discovery? Drug Discov Today 8: 445-450.

53. Rudolph U, Möhler H (2004) Analysis of GABAA receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. Annu Rev Pharmacol Toxicol 44: 475-498.

54. Rowlett JK, Platt DM, Lelas S, Atack JR, Dawson GR (2005) Different GABAA receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drug in primates. Proc Natl Acad of Sci USA 102: 915-920.

55. Wikinski S, Lombardo M, Medina JH, Rubio MC (1994) Lack of anxiolytic effect of diazepam in pre-anaesthetic medication. Br J Anaesth 72: 694-696.

56. Ravenelle R, Neugebauer NM, Niedzielak T, Donaldson ST (2014) Sex differences in diazepam effects and parvalbumin-positive GABA neurons in trait anxiety Long Evans rats. Behav Brain Res 270: 68-74.

57. Guimarães FS, Kohem CL, Gus G, Fillmann HS, de-Vecino MC, et al. (1989) A simple simulated public speaking test for evaluating anxiolytic drugs. Braz J Med Biol Res 22: 1083-1089.

58. Albus M, Zellner A, Bondy B, Müller-Spahn F, Engel R, et al. (1989) Influence of CGP 361A, propranolol and diazepam on autonomous reactions to different stressors. Prog Neuropsychopharmacol Biol Psychiatry 13: 87-97.

59. Benschop RJ, Jacobs R, Sommer B, Schürmeyer TH, Raab JR, et al. (1996) Modulation of the immunologic response to acute stress in humans by beta-blockade or benzodiazepines. FASEB J 10: 517-524.

60. Castiglia YM, Boggiani E, de Macedo LA, Asada L, Guizilini RH, et al. (1987) Comparative study of diazepam and lorazepam by oral route, associated with meperidine in preanesthetic medication. Rev Paul Med 105: 130-133.

61. Stewart SH, Westra HA (2002) Benzodiazepine side-effects: from the bench to the clinic. Curr Pharm Des 8: 1-3.

62. Cogo K, Bergamaschi CC, Yatsuda R, Volpato MC, Andrade ED (2006) Sedação consciente com benzodiazepínicos em odontologia. Rev Odontol Univ São Paulo 18: 181-188.

63. Donaldson M, Gizzarelli G, Chanpong B (2007) Oral sedation: a primer on anxiolysis for the adult patient. Anesth Prog 54: 118-128.

64. Ogle OE, Hertz MB (2012) Anxiety control in the dental patient. Dent Clin North Am 56: 1-16, vii.

65. Correa-De-Araujo R (2006) Serious gaps: how the lack of sex/gender-based research impairs health. J Womens Health (Larchmt) 15: 1116-1122.

66. Geller SE, Adams MG, Carnes M (2006) Adherence to federal guidelines for reporting of sex and race/ethnicity in clinical trials. J Womens Health (Larchmt) 15: 1123-1131.

67. Cafferata GL, Kasper J, Bernstein A (1983) Family roles, structure, and stressors in relation to sex differences in obtaining psychotropic drugs. J Health Soc Behav 24: 132-143.

68. Kessler RC, Brown RL, Broman CL (1981) Sex differences in psychiatric help-seeking: evidence from four large-scale surveys. J Health Soc Behav 22: 49-64.

69. Yonkers KA, Kando JC, Cole JO, Blumenthal S (1992) Gender differences in pharmacokinetics and pharmacodynamics of psychotropic medication. Am J Psychiatry 149: 587-595.