The involvement of noradrenergic mechanisms in the suppressive effects of diazepam on the hypothalamic-pituitary-adrenal axis activity in female rats

**Aim** To elucidate the involvement of noradrenergic system in the mechanism by which diazepam suppresses basal hypothalamic-pituitary-adrenal (HPA) axis activity.

**Methods** Plasma corticosterone and adrenocorticotropic hormone (ACTH) levels were determined in female rats treated with diazepam alone, as well as with diazepam in combination with clonidine (α2-adrenergic receptor agonist), yohimbine (α2-adrenergic receptor antagonist), alpha-methyl-p-tyrosine (α-MPT, an inhibitor of catecholamine synthesis), or reserpine (a catecholamine depleting drug) and yohimbine.

**Results** Diazepam administered in a dose of 2.0 mg/kg suppressed basal HPA axis activity, ie, decreased plasma corticosterone and ACTH levels. Pretreatment with clonidine or yohimbine failed to affect basal plasma corticosterone and ACTH concentrations, but abolished diazepam-induced inhibition of the HPA axis activity. Pretreatment with α-MPT, or with a combination of reserpine and yohimbine, increased plasma corticosterone and ACTH levels and prevented diazepam-induced inhibition of the HPA axis activity.

**Conclusion** The results suggest that α2-adrenergic receptors activity, as well as intact presynaptic noradrenergic function, are required for the suppressive effect of diazepam on the HPA axis activity.
Benzodiazepines are used for their anxiolytic, sedative-hypnotic, muscle relaxant, and anticonvulsant properties in the treatment of a variety of neuropsychiatric disorders (1,2), including anxiety and depression, which are often related to disturbances in the activity of hypothalamic-pituitary-adrenal (HPA) axis (3,4). Although these drugs exert most of their pharmacological effects via γ-aminobutyric acid_A (GABA_A) receptors (5,6), benzodiazepine administration has been associated with alterations in neuroendocrine function both in experimental animals and humans (7-9). However, even after years of extensive studies, the complex mechanisms by which these widely used drugs produce their effects on the HPA axis are still not known.

Although most of the previous studies have demonstrated that classical benzodiazepines such as diazepam decrease the HPA axis activity in stressful contexts (10-14), under basal conditions they have been shown to stimulate (9,11,15-18), inhibit (15,19-22), and not affect (17,23-25) the HPA axis activity. Such diverse results might be related to several factors such as the dose and gender (15,16,20,21,26-28), or may also be a consequence of the net effect of non-selective benzodiazepines on the various GABA_A receptor isoforms (9).

Our previous studies demonstrated that while diazepam (1 mg/kg) produced no change in plasma corticosterone levels in male rats (15,20), it decreased basal levels of corticosterone in female rats (15,26). However, although diazepam inhibited the HPA axis activity of female rats following administration of lower doses (1 or 2 mg/kg) (15,20), it stimulated the HPA axis activity following administration of high doses (10 mg/kg) (15,16,26). Moreover, whereas the suppressive effect of the lower doses of diazepam (2.0 mg/kg) on the HPA axis activity in female rats involves the GABA_A receptor complex (21), increases in corticosterone levels by a higher dose of diazepam (10 mg/kg) do not involve the stimulation of GABA_A receptors (16). In addition, stimulatory effect of 10 mg/kg diazepam on the HPA axis activity in rats seems not to be mediated by the benzodiazepine/GABA/channel chloride complex or by peripheral benzodiazepine receptors, but rather by a cyclic adenosine monophosphate (AMP)-dependent mechanism (18).

Since our previous results suggested that the effect of a high dose of diazepam on the activity of the HPA axis in female rats might be due to a blockade of α_2-adrenergic receptors (16), the aim of this study was to elucidate whether noradrenergic system also has a modulatory role in the inhibitory effect of 2.0 mg/kg diazepam on basal plasma adrenocorticotropic hormone (ACTH) and corticosterone levels in female rats.

METHODS

Animals

Female adult Wistar rats (180-200 g), bred in our Institute, were housed under controlled conditions, with standard light-dark cycle and food and water freely available. The rats were caged in groups of five. Each experiment included approximately 30-35 rats and was performed twice. The total number of animals used in the study was 260. To minimize circadian variability of plasma ACTH and corticosterone levels, all experiments were performed between 8.00 and 12.00 AM. The only exception was the experiment with reserpine in which reserpine was administered during the working and day-light time on the day prior to the experiment, so that the administration of yohimbine and diazepam, as well as sacrifice could be performed until 12.00 AM of the next day. The phases of the estrous cycle of female rats were not monitored or synchronized prior to experiments, since we presumed on the basis of previous studies (29-31) that the sexual cycle of female rats used in our study would not significantly affect diazepam-induced reductions of basal plasma corticosterone or ACTH levels. Before the experiments, all animals were adapted to handling and intraperitoneal (ip) injecting for 7 days. All animal care and experimental procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1996), and with the Croatian law on animal welfare. Ethical approval was received from the Ministry of Agriculture – Veterinary Directorate and Ruđer Bošković Institute Ethics Review Board.

Drugs

Diazepam (Krka, Novo Mesto, Slovenia) and clonidine (Boehringer Ingelheim on Rhein, Germany) were dissolved in 0.1 N HCl, followed by saline. Yohimbine (Sigma Chemical Co, St. Louis, MO, USA) and α-methyl-p-tyrosine (α-MPT, Sigma) were dissolved in distilled water. Reserpine (Sigma) was suspended in glacial acetic acid and 5.5% glucose was added to the final volume. All drugs and corresponding vehicles were prepared fresh, and after pH adjustment injected ip in a volume of 1 mL/100 g bw.

Experimental procedure

Reserpine (10.0 mg/kg), α-methyl-p-tyrosine (400.0 mg/kg), yohimbine (0.5 and 3.0 mg/kg), clonidine (0.01 and 0.5 mg/kg), diazepam (2.0 mg/kg), or cor-
responding vehicles were injected ip 15h, 4h, 90-minute, 35-minute, and 30-minute prior to sacrifice, respectively. The doses and time points of the drugs used in the study were selected based on the previous studies conducted in our laboratory and those reported in literature. Namely, since the results of our dose-response study (data available on request) revealed that the most significant decrease in plasma corticosterone levels was produced by the administration of 2.0 mg/kg diazepam, this dose was further used in our experiments. The two doses of clonidine (0.01 and 0.5 mg/kg) were used with the aim to differentiate between presynaptic and postsynaptic effects of this drug (32,33). Yohimbine was administered in two doses (0.5 mg/kg and 3 mg/kg), which have been already used in various studies (34,35). In order to ensure the complete inhibition of tyrosine hydroxylase, the enzyme responsible for catecholamine synthesis, alpha-methyl-p-tyrosine (α-MPT) was administered in a high dose (400.0 mg/kg), previously reported to inhibit catecholamine synthesis (36). To find out whether diazepam affects the HPA axis activity in conditions which completely eliminate the noradrenergic transmission, rats were pretreated with yohimbine (3.0 mg/kg) in a combination with reserpine administered in a high dose (10 mg/kg), already used for catecholamine depletion by other authors (37). Animals were sacrificed by decapitation with a guillotine. Trunk blood was collected in prechilled tubes containing EDTA (1 mg/mL of blood), centrifuged (4°C, 10-minute, 1250 g) and plasma was stored at -20°C. Corticosterone levels were determined in plasma samples of 500 μL by a slight modification of the fluorometric method (21). ACTH levels were measured directly, without prior extraction, in a 200 µL of plasma, by a radio-immunoassay using commercially available ACTH-K-PR kit (CIS bioindustries, Gif sur Yvette, France).

Data analysis

Results were expressed as percents ± standard error of the mean of the values in control animals. Statistical evaluation of the results was done with GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA, USA) by using one-way analysis of variance (ANOVA) followed by Tukey test. P-values of <0.05 were considered significant.

RESULTS

The administration of 2.0 mg/kg of diazepam to female rats significantly reduced plasma corticosterone (P < 0.001) and ACTH (P < 0.01) levels. Both doses of clonidine (0.01 and 0.5 mg/kg) failed to affect plasma corticosterone and ACTH levels in control rats, but clonidine administered in a dose of 0.5 mg/kg antagonized diazepam-induced decrease of both hormones (P < 0.05) (Figure 1A and 1B). The administration of yohimbine in two doses (0.5 mg/kg and 3 mg/kg) elicited no effect on basal plasma corticosterone and ACTH levels. Although diazepam-induced reduction of plasma corticosterone levels (P < 0.001) was reversed already with 0.5 mg/kg yohimbine (P < 0.01) (Figure 2A), the higher (3.0 mg/kg) dose of yohimbine was required to counteract (P < 0.001) the effect of diazepam (P < 0.01) on plasma ACTH levels (Figure 2B). The administration of α-MPT significantly increased (P < 0.001) plasma corticosterone and ACTH levels (Figure 3A and 3B). Although diazepam significantly decreased (P < 0.001) plasma corticosterone and ACTH

![Figure 1. The effect of clonidine on diazepam-induced decrease of plasma corticosterone (A) and adrenocorticotropic hormone (ACTH) (B) concentrations. Clonidine (0.01 and 0.5 mg/kg) or its vehicle and diazepam (2.0 mg/kg) or its vehicle were injected ip 35 and 30 minutes, respectively, prior to sacrifice. The results are expressed as percents ± standard error of the mean of the values in control animals treated with the corresponding vehicles. The number of animals per group was 12 for determination of corticosterone levels and 7 for determination of ACTH levels. *P < 0.01; †P < 0.001 vs the control vehicles-treated group; ‡P < 0.05 vs diazepam-treated group (one-way ANOVA followed by Tukey test).](image-url)
levels in control rats, it was unable to diminish the increase of corticosterone and ACTH in the rats pretreated with α-MPT (Figure 3A and 3B). Plasma corticosterone levels were similar between rats treated with α-MPT alone or α-MPT in combination with diazepam, but the combination of α-MPT and diazepam had an additive effect on plasma ACTH levels (P<0.01). The combined treatment with reserpine and yohimbine induced a significant increase (P<0.001) in plasma corticosterone and ACTH levels (Figure 4A and 4B). Diazepam significantly decreased plasma corticosterone and ACTH levels in control rats (P<0.01), but could not suppress the elevated concentrations of both hormones in the rats pretreated with reserpine and yohimbine (Figure 4A and 4B).

**FIGURE 2.** The effect of yohimbine on diazepam-induced decrease of plasma corticosterone (A) and adrenocorticotropic hormone (ACTH) (B) concentrations. Yohimbine (0.5 and 3.0 mg/kg) or its vehicle and diazepam (2.0 mg/kg) or its vehicle were injected i.p. 90 and 30 minutes, respectively, prior to sacrifice. The results are expressed as percents ± standard error of the mean of the values in control animals treated with the corresponding vehicles. The number of animals per group was 12 for determination of corticosterone levels and 7 for determination of ACTH levels. *P<0.01; †P<0.01 vs the control vehicles-treated group; ‡P<0.01; §P<0.001 vs diazepam-treated group (one-way ANOVA followed by Tukey test).

**FIGURE 3.** The effect of α-methyl-p-tyrosine (α-MPT) on diazepam-induced decrease of plasma corticosterone (A) and adrenocorticotropic hormone (ACTH) (B) concentrations. Alpha-methyl-p-tyrosine (400.0 mg/kg) or its vehicle and diazepam (2.0 mg/kg) or its vehicle were injected i.p. 240 and 30 minutes, respectively, prior to sacrifice. The results are expressed as percents ± standard error of the mean of the values in control animals treated with the corresponding vehicles. The number of animals per group was 15 for determination of corticosterone levels and 9 for determination of ACTH levels. *P<0.01 or P<0.05 vs the control vehicles-treated group; †P<0.001 vs the control vehicles-treated and diazepam-treated group; ‡P<0.01 vs α-methyl-p-tyrosine-treated group (one-way ANOVA followed by Tukey test).

**DISCUSSION**

The observed inhibitory effect of 2.0 mg/kg diazepam on the HPA axis activity agrees with our previous studies that used the same (21) or similar (20,26) doses and timing of diazepam administration, but disagrees with some results which demonstrated no changes in the levels of corticosterone induced by diazepam treatment (23). Some of the discrepancies considering the effects of diazepam on the basal HPA activity (stimulation, inhibi-
tion, or no effect) could be ascribed to the differences in sex (15,20,28,38), age (13,39), basal vs stressful conditions (10,13,38), or the strain of the animals used (40).

The lack of an effect of clonidine on plasma corticosterone and ACTH levels observed in our study agrees with other data (41,42). The finding that pretreatment with clonidine counteracted the diazepam-induced suppression of corticosterone and ACTH levels suggested that α₂-adrenoreceptors are probably involved in the inhibition of the HPA axis produced by diazepam. However, yohimbine also reversed the inhibitory effect of diazepam on plasma corticosterone and ACTH levels, and these results are in line with a study showing that yohimbine prevented decreases in both of these hormones induced by the intraventricular administration of GABA (43).

The fact that treatments with α₂-adrenoreceptor agonist clonidine and α₂-adrenoreceptor antagonist yohimbine had the same effects, ie, that both abolished diazepam-induced inhibition of the HPA axis activity, might be explained by the pre- vs post-synaptic activities of these drugs. Namely, while the effect of clonidine administered in a dose of 0.5 mg/kg is likely to be produced by activation of postsynaptic α₂-adrenoreceptors (32), the observed effect of yohimbine is probably due to the blockade of inhibitory presynaptic α₂-adrenergic receptors, resulting in increased norepinephrine release (44). However, as the action of clonidine and yohimbine could be mediated by the activation or by the blockade of α₂-adrenoreceptors (32,41,45), and as the drugs used are not absolutely selective, other more receptor-distinctive agonists and/or antagonists might be useful in revealing the potential involvement of these receptors in diazepam-induced suppression of the HPA axis.

The results showing that diazepam was unable to counteract the enhancement of corticosterone and ACTH levels elicited by α-MPT (an inhibitor of tyrosine hydroxylase, the enzyme responsible for catecholamine synthesis), suggested that diazepam suppresses the HPA axis activity by an action that also requires the intact presynaptic noradrenergic function. Moreover, treatment with combination of reserpine (a catecholamine depleting drug) and yohimbine (α₂-adrenoreceptor antagonist), which creates a condition that diminishes noradrenergic transmission, completely abolished the inhibitory effect of diazepam on plasma concentrations of corticosterone and ACTH. These results indicate that diazepam cannot exert any inhibitory influence when HPA axis is stimulated by drugs affecting noradrenergic neurotransmission such as α-MPT and reserpine, already reported to enhance corticosterone and/or ACTH levels (41,46,47).

To our knowledge, the present study was the first to demonstrate that besides central benzodiazepine receptors, the part of GABAₐ receptor complex (21), α₂-adrenoreceptors activity, and intact presynaptic noradrenergic function were required for the suppressive effect of 2.0 mg/kg di-

![FIGURE 4. The effect of combined pretreatment with reserpine and yohimbine on diazepam-induced decrease of plasma corticosterone (A) and adrenocorticotropic hormone (ACTH) (B) concentrations. Reserpine (10.0 mg/kg) or its vehicle, yohimbine (3.0 mg/kg) or its vehicle, and diazepam (2.0 mg/kg) or its vehicle were injected ip 15 hours, 90 minutes, and 30 minutes, respectively, prior to sacrifice. The results are expressed as percents ± standard error of the mean of the values in control animals treated with the corresponding vehicles. The number of animals per group was 14 for determination of corticosterone levels and 7 for determination of ACTH levels. *P < 0.01 vs the control vehicles-treated group; †P < 0.001 vs the control vehicles-treated and diazepam-treated group (one-way ANOVA followed by Tukey test).](image-url)
Diazepam, noradrenaline, and HPA axis activity

The activity of the HPA axis in female rats. The suggestion that diazepam affects the HPA axis via noradrenergic system (Figure 5) also agrees with the previously shown interactions between diazepam and noradrenergic function (48-50).

However, the fact that diazepam (1 mg/kg) produced no change in basal plasma corticosterone levels in male rats (15,20) points out differences in the regulatory mechanisms of the HPA axis activity between males and females (10,30,38,51,52). These findings are in line with the studies demonstrating that the effects of benzodiazepine exposure on corticosterone and ACTH levels, are influenced by gender-related factors (27,30). Namely, gonadal steroid hormones including estrogens and androgens have been shown to modulate the GABA/BZ receptors or their responses, as well as the HPA axis activity implicated in the different stress responses between genders (27,30,53,54). Moreover, sexually dimorphic alternations in CRH system and HPA responses to stress are suggested to play a role in the etiology of affective and anxiety-related disorders and may be affected by both estrogen and progesterone (27).

Such neuropsychiatric conditions have been also associated with perturbations of endogenous neurosteroids within the CNS (55,56). Neurosteroid levels are dynamically regulated in response to a number of physiological states, such as stress, development, puberty, pregnancy, menopause, and during the ovarian cycle (57,58). In addition, it has been demonstrated that treatment with certain psychoactive drugs can affect neurosteroid content in the brain (59,60). Namely, certain benzodiazepines including diazepam, besides enhancing the GABA<sub>A</sub> receptor function, have been shown to promote the synthesis of neurosteroids (61,62). Hence, in agreement with the known role of neurosteroids as potent positive allosteric modulators of GABA action at GABA<sub>A</sub> receptors (62), the effects of diazepam might be potentiated by endogenous neurosteroids (63). As neurosteroids participate in the homeostatic regulation of HPA axis under basal as well as stress conditions (64), they could also contribute to diazepam-induced suppression of HPA axis activity, by additionally decreasing ACTH and corticosterone levels, especially during stress when rapid elevation of neurosteroid levels occurs (65,66).

In conclusion, our present and previous (16) data suggested that noradrenergic system might have an important modulatory role in the suppressive as well as in the stimulatory effects of diazepam on the HPA axis activity in female rats. As disturbances in the HPA axis activity are found in a variety of psychiatric disorders that are treated with ben-

![FIGURE 5](image-url)
zodiazepines, including depression, insomnia, and anxiety (3,4), we could speculate that the therapeutic effects of benzodiazepines might be achieved not only by their well-known mechanisms, but also through modulation of the HPA axis activity via noradrenergic system. Considering the fact that mood and anxiety disorders as well as use of benzodiazepines are highly prevalent among women (28,67,68), these findings might be relevant for the therapeutic implications in female population. This might be particularly the case in the combined treatment of benzodiazepines and antidepressants, such as tricyclic antidepressants, noradrenaline reuptake inhibitors, and serotonin-noradrenaline reuptake inhibitors (69,70). These combinations are especially common in the treatment of co-morbid depression and anxiety. Since some antidepressant drugs might affect the noradrenergic neurotransmission, which seems to be involved in the opposite effects of different doses of diazepam on the HPA axis activity, a special attention should be given to adjustment of particular doses of benzodiazepines and antidepressants, when used in combination.

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Competing interests All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization or entity (private or public); no relationships or activities that could appear to have influenced the submitted work.

References

1 Bateson AN. Basic pharmacologic mechanisms involved in benzodiazepine tolerance and withdrawal. Curr Pharm Des. 2002;8:5-21. Medline:11812247 doi:10.2174/1381612023396681

2 Tan KR, Rudolph U, Lüscher C. Hooked on benzodiazepines: GABAA receptor subtypes and addiction. Trends Neurosci. 2011;34:188-97. Medline:21533710 doi:10.1016/j.tins.2011.01.004

3 Arborelius L, Owens MJ, PLOTSKY PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinol. 1999;162:1-12. Medline:9854171 doi:10.1677/joe.0.160001

4 Hollsboer F. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology. 2000;23:477-501. Medline:11027914 doi:10.1016/S0893-133X(00)00159-7

5 Korpi ER, Gründler G, Lüddens H. Drug interactions at GABAA receptors. Prog Neurobiol. 2002;67:113-59. Medline:12126558 doi:10.1016/S0301-0082(02)00013-8

6 Olsen RW, Sieghart W. GABA A receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology. 2009;56:141-8. Medline:18760291 doi:10.1016/j.neuropharm.2008.07.045

7 Arvat E, Giordano R, Grotti S, Ghigo E. Benzodiazepines and anterior pituitary function. J Endocrinol Invest. 2002;25:735-47. Medline:12240908

8 Heberlein A, Bleich S, Kornhuber J, Hillemacher T. Neuroendocrine pathways in benzodiazepine dependence: new targets for research and therapy. Hum Psychopharmacol. 2008;23:171-81. Medline:18088080 doi:10.1002/hup.911

9 Mikkel森 JD, Soderman A, Kiss A, Mirza N. Effects of benzodiazepine receptor agonists on the hypothalamic-pituitary-adrenocortical axis. Eur J Pharmacol. 2005;519:223-30. Medline:16125698 doi:10.1016/j.ejphar.2005.06.049

10 Pericic D, Pivac N. Sex differences in conflict behaviour and in plasma corticosterone levels. J Neural Transm Gen Sect. 1995;101:213-21. Medline:8695051 doi:10.1007/BF01271558

11 Kalman BA, Kim PJ, Cole MA, Chi MS, Spencer RL. Diazepam attenuation of restraint stress-induced corticosterone levels is enhanced by prior exposure to repeated restraint. Psychoneuroendocrinology. 1997;22:349-60. Medline:9279940 doi:10.1016/S0306-4530(97)00026-7

12 Carrasco GA, Van de Kar LD. Neuroendocrine pharmacology of stress. Eur J Pharmacol. 2003;463:235-72. Medline:12600714 doi:10.1016/S0014-2999(03)01285-8

13 Pomara N, Willoughby LM, Sidtis JJ, Cooper TB, Greenblatt DJ. Cortisol response to diazepam: its relationship to age, dose, duration of treatment, and presence of generalized anxiety disorder. Psychopharmacology (Berl). 2005;178:1-8. Medline:15338100 doi:10.1007/s00213-004-1974-8

14 Fries E, Hellhammer DH, Hellhammer J. Attenuation of the hypothalamic-pituitary-adrenal axis responsivity to the Trier Social Stress Test by the benzodiazepine alprazolam. Psychoneuroendocrinology. 2006;31:1278-88. Medline:17097811 doi:10.1016/j.psyneuen.2006.09.009

15 Lakic N, Pericic D, Manev H. Sex differences in the plasma corticosterone response of rats to diazepam and picrotoxin. Period Biol. 1984;87:417.

16 Lakic N, Pericic D, Manev H. Mechanisms by which picrotoxin and a high dose of diazepam elevate plasma corticosterone levels. Neuroendocrinology. 1986;43:331-5. Medline:3736781
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17 De Boer SF, Van Der Gugten J, Slangen JL. Effects of chlordiazepoxide, flumazenil and DMCM on plasma catecholamine and corticosterone concentrations in rats. Pharmacol Biochem Behav. 1991;38:13-9. Medline:1850134 doi:10.1016/0091-3057(91)90583-N

18 Vargas ML, Abella C, Hernandez J. Diazepam increases the hypothalamic-pituitary-adrenocortical (HPA) axis activity by a cyclic AMP-dependent mechanism. Br J Pharmacol. 2001;133:1355-61. Medline:11498522 doi:10.1038/sj.bjp.0704201

19 Kalogeras KT, Calogero AE, Kuribayashi T, Khan I, Gallucci WT, Kling MA, et al. In vitro and in vivo effects of the triazolobenzodiazepine alprazolam on hypothalamic-pituitary-adrenal function: pharmacological and clinical implications. J Clin Endocrinol Metab. 1990;70:1462-71. Medline:2159487 doi:10.1210/jcem-70-5-1462

20 Pericic D, Manev H, Lakic N. Sex differences in the response of rats to drugs affecting GABAergic transmission. Life Sci. 1985;36:541-7. Medline:2982069 doi:10.1016/0028-3908(85)90635-6

21 Pivac N, Pericic D. Inhibitory effect of diazepam on the activity of the hypothalamic-pituitary-adrenal axis in female rats. J Neural Transm Gen Sect. 1993;92:173-86. Medline:8396396 doi:10.1007/BF01244876

22 Skelton KH, Nemeroff CB, Knight DL, Owens MJ. Chronic administration of the triazolobenzodiazepine alprazolam produces opposite effects on corticotropin-releasing factor and urocortin neuronal systems. J Neurosci. 2000;20:1240-8. Medline:10648728

23 Matheson GK, Gage D, White G, Dixon V, Gipson D. A comparison of the effects of buspirone and diazepam on plasma corticosterone levels in rat. Neuropharmacology. 1988;27:823-30. Medline:3216961 doi:10.1016/0028-3908(88)90098-6

24 Olivier B, Zethof T, Pattij T, van Boogaert M, van oorschot R, Leahy C, et al. Stress-induced hyperthermia and anxiety: pharmacological validation. Eur J Pharmacol. 2003;463:117-32. Medline:12600705 doi:10.1016/S0014-2999(03)01326-8

25 Broadbear JH, Winger G, Woods JH. Self-administration of methohexital, midazolam and ethanol: effects on the pituitary-adrenal axis in rhesus monkeys. Psychopharmacology (Berl). 2005;178:83-91. Medline:15322724 doi:10.1007/s00213-004-1986-4

26 Pericic D, Lakic N, Manev H. Effect of diazepam on plasma corticosterone levels. Psychopharmacology (Berl). 1984;83:79-81. Medline:6429704 doi:10.1007/BF00427427

27 Wilson MA, Biscardi R, Smith MD, Wilson SP. Effects of benzodiazepine agonist exposure on corticotropin-releasing factor content and hormonal stress responses: divergent responses in male and ovariecctomized female rats. J Pharmacol Exp Ther. 1996;278:1073-82. Medline:8819488

28 Pomara N, Willoughby LM, Ritchie JC, Sidtis JJ, Greenblatt DJ, Nemeroff CB. Sex-related elevation in cortisol during chronic treatment with alprazolam associated with enhanced cognitive performance. Psychopharmacology (Berl). 2005;182:414-9. Medline:16001108 doi:10.1007/s00213-005-0086-2

29 Viau V, Meany MJ. Variations in the hypothalamic-pituitary-adrenal response to stress during the estrous cycle in the rat. Endocrinology. 1991;129:2503-11. Medline:1657578 doi:10.1210/endo-129-5-2503

30 Wilson MA, Biscardi R. Sex differences in GABA/benzodiazepine receptor changes and corticosterone release after acute stress in rats. Exp Brain Res. 1994;101:297-306. Medline:7843316 doi:10.1007/BF00228750

31 Wilson MA. Influences of gender, gonadectomy, and estrous cycle on GABA/BZ receptors and benzodiazepine responses in rats. Brain Res Bull. 1992;29:165-72. Medline:1356068 doi:10.1007/9230(92)90022-P

32 Gonzalez ML, Milanes MV, Martinez-Piñero MG, Marin MT, Vargas ML. Effects of intracerebroventricular clonidine on the hypothalamic noradrenaline and plasma corticosterone levels of opiate naive rats and after naloxone-induced withdrawal. Brain Res. 1994;647:199-203. Medline:7922496 doi:10.1016/0006-8993(94)91318-8

33 Franovicz JS, Amsten AF. Treatment with the noradrenergic alpha-2 agonist clonidine, but not diazepam, improves spatial working memory in normal young rhesus monkeys. Neuropsychopharmacology. 1999;21:611-21. Medline:10516957 doi:10.1016/S0893-133X(99)00060-3

34 Archer T, Fredriksson A. Effects of clonidine and alpha-adrenoceptor antagonists on motor activity in DSP4-treated mice I: dose-, time- and parameter-dependency. Neurotox Res. 2000;1:235-47. Medline:12835092 doi:10.1007/BF03033254

35 Haapanilma A, Leino T, Heinonen E. The alpha 2-adrenoceptor antagonist atipamezole potenitizes anti-Parkinsonian effects and can reduce the adverse cardiovascular effects of dopaminergic drugs in rats. Naunyn Schmiedebergs Arch Pharmacol. 2003;368:342-51. Medline:14566451 doi:10.1007/s00210-003-0827-2

36 Ponzo OJ, Seilicovich A, Rondina D, Piseria D, Calcagno ML, Scacchi P. Aproteic diet decreases hypothalamic catecholamine turnover in adult male rats. Brain Res. 2000;871:44-9. Medline:10882781 doi:10.1016/S0006-8993(00)02419-7

37 Fredriksson A, Archer T. Effects of clonidine and alpha-adrenoceptor antagonists on motor activity in DSP4-treated mice II: interactions with apomorphine. Neurotox Res. 2000;1:249-59. Medline:12835093 doi:10.1007/BF03033255

38 Pericic D, Pivac N. Effect of diazepam on conflict behavior and on plasma corticosterone levels in male and female rats. Naunyn Schmiedebers Arch Pharmacol. 1996;353:369-76. Medline:8935702

39 Meaney MJ, Atken DH, Sharma S, Vnau V, Basal ACHT, corticosterone and corticosterone-binding globulin levels over the diurnal cycle, and age-related changes in hippocampal type I and
type II corticosteroid receptor binding capacity in young and aged, handled and nonhandled rats. Neuroendocrinology. 1992;55:204-13. Medline:1320217 doi:10.1159/000126116

40 Otzi MS, Vanhaaert AD, Sutanto W, Dekloet ER. Corticosterone, brain mineralocorticoid receptors (MRS) and the activity of the hypothalamic-pituitary-adrenal (HPA) axis - the Lewis rat as an example of increased central MR capacity and a hyporesponsive HPA axis. Psychoneuroendocrinology. 1993;20:655-75. Medline:8584606 doi:10.1016/0306-4530(93)90003-7

41 Kovacs KJ, Makara GB. Factors from the paraventricular nucleus mediate inhibitory effect of alpha-2-adrenergic drugs on ACTH secretion. Neuroendocrinology. 1993;57:346-50. Medline:8389998 doi:10.1159/000126378

42 Jezova-Repcekova D, Vigas M, Kulifajova A. Effects of phentolamine on corticosterone secretion: involvement of the conscious rats. Endocrinol Exp. 1978;12:3-8. Medline:305843

43 Miguez I, Aldenguide MA. Effect of gamma-aminobutyric acid on corticosterone secretion: Involvement of the noradrenergic system. Life Sci. 1990;46:875-80. Medline:1690835 doi:10.1016/0024-3205(90)90117-A

44 Majewski H, Rump LC, Hedler L, Starke K. Effects of alpha 1- and alpha 2-adrenoceptor blocking drugs on noradrenaline release rate anaesthetized rabbits. J Cardiovasc Pharmacol. 1983;5:703-11. Medline:6195454 doi:10.1093/clinph/57.3.703

45 Millan MJ, Newman-tancredi A, Audinot V, Cussac D, Lejeune F, Londeo P, Rupprecht R, Manieri GA, Bernardi G, Romeo E, Pasini S. Regulatory mechanisms underlying the hypothalamic-pituitary-adrenal axis and the female reproductive system. Ann N Y Acad Sci. 1997;816:42-56. Medline:9238254 doi:10.1111/j.1749-6632.1997.tb52128.x

46 Smythe GA, Bradshaw JE. Different acute effects of the tyrosine hydroxylase inhibitors a-metil-para-tyrosine and 3-iodo-l-tyrosine on hypothalamic noradrenaline activity and adrenocorticotoxphrin release in the rat. Aust J Biol Sci. 1983;36:519-23. Medline:6144300

47 Suda T, Tajiri T, Aoki Y, Takeshita I, Otsuka K, Nishikawa Y. Psychological stress-induced increases in noradrenaline release in rat brain regions are attenuated by diazepam, but not by morphine. Pharmacol Biochem Behav. 1991;39:191-5. Medline:1924502 doi:10.1016/0091-3057(91)90420-7

48 Pericic D, Manev H, Boronic M, Poljak-Blazi M, Lakic N. Effect of diazepam on brain neurotransmitters, plasma corticosterone and the immune system of the rats. Ann N Y Acad Sci. 1987;496:450-8. Medline:3474983 doi:10.1111/j.1749-6632.1987.tb35801.x

49 Finlay JM, Zigmond MJ, Abercrombie ED. Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: Effect of diazepam. Neuroscience. 1995;64:619-28. Medline:7715775 doi:10.1016/0306-4522(94)00331-x

50 Tanaka M, Tsuda A, Yokoo H, Yoshida M, Mizoguchi K, Shimizu T. Psychological stress-induced increases in noradrenaline release in rat brain regions are attenuated by diazepam, but not by morphine. Pharmacol Biochem Behav. 1991;39:191-5. Medline:1924502 doi:10.1016/0091-3057(91)90420-7

51 Seeman TE, Singer B, Wilkinson CW, McEwen B. Gender differences in age-related changes in HPA axis reactivity. Psychoneuroendocrinology. 2001;26:225-40. Medline:11166486 doi:10.1016/s0306-4530(00)00043-3

52 Kudielka BM, Kirschbaum C. Sex differences in HPA axis responses to stress: a review. Biol Psychol. 2005;69:113-32. Medline:15740829 doi:10.1016/j.biopsycho.2004.11.009

53 Magiakou MA, Mastorakos G, Webster E, Chrousos GP. The hypothalamic-pituitary-adrenal axis and the female reproductive system. Ann N Y Acad Sci. 1997;816:42-56. Medline:9238254 doi:10.1111/j.1749-6632.1997.tb52128.x

54 Kageyama K, Suda T. Regulatory mechanisms underlying corticotropin-releasing factor gene expression in the hypothalamus. Endocr J. 2009;56:335-44. Medline:19352056 doi:10.1507/endocrj.K09e-075

55 Longone P, Rupprecht R, Manieri GA, Bernardi G, Romeo E, Pasini A. The complex roles of neurosteroids in depression and anxiety disorders. Neurochem Int. 2008;52:596-601. Medline:17996986 doi:10.1016/j.neuint.2007.10.001

56 Schüle C, Eser D, Baghai TC, Nothdurfter C, Kessler JS, Rupprecht R. Neuroactive steroids in affective disorders: target for novel antidepressant or anxiolytic drugs? Neuroscience. 2011;191:55-77. Medline:21439354 doi:10.1016/j.neuroscience.2011.03.025

57 Pearson Murphy BE, Steinberg SI, Hu FY, Allison CM. Neuroactive ring A-reduced metabolites of progesterone in human plasma during pregnancy: elevated levels of 5 alpha-dihydroprogesterone in depressed patients during the latter half of pregnancy. J Clin Endocrinol Metab. 2001;86:5981-7. Medline:11739473 doi:10.1210/jc.86.12.5981

58 Maguire J, Mody I. Steroid hormone fluctuations and GABA(A) receptor plasticity. Psychoneuroendocrinology. 2009;34:584-90. Medline:19632051 doi:10.1016/j.psyneuen.2009.06.019

59 Uzunova V, Sheline Y, Davis JM, Rasmussen A, Uzunov DP, Costa E, et al. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proc Natl Acad Sci U S A. 1998;95:3239-44. Medline:9501247 doi:10.1073/pnas.95.6.3239

60 Barbaccia ML, Colombo G, Affricano D, Calai MA, Vaccia G, Melis S, et al. GABA(B) receptor-mediated increase of neurosteroids by gamma-hydroxybutyric acid. Neuropharmacology. 2002;42:782-91. Medline:12015204 doi:10.1016/s0028-3908(02)00026-6

61 Tokuda K, O’Dell KA, Izu Y, Zoromski CF. Midazolam inhibits hippocampal long-term potentiation and learning through dual central and peripheral benzodiazepine receptor activation and neurosteroidogenesis. J Neurosci. 2010;30:16788-95. Medline:21159950 doi:10.1523/JNEUROSCI.4101-10.2010
62 Gunn BG, Brown AR, Lambert JJ, Belelli D. Neurosteroids and GABA(A) receptor interactions: a focus on stress. Front Neurosci. 2011;5:131. Medline:22164129 doi:10.3389/fnins.2011.00131
63 Paronis CA. Modulating GABA modulators. Br J Pharmacol. 2006;147:237-8. Medline:16331288 doi:10.1038/sj.bjp.0706552
64 Mody I, Maguire J. The reciprocal regulation of stress hormones and GABA(A) receptors. Front Cell Neurosci. 2011;6:4. Medline:22319473
65 Purdy RH, Morrow AL, Moore PH Jr, Paul SM. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. Proc Natl Acad Sci U S A. 1991;88:4553-7. Medline:1852011 doi:10.1073/pnas.88.10.4553
66 Barbaccia ML, Roscetti G, Trabucchi M, Mostallino MC, Concas A, Purdy RH, et al. Time-dependent changes in rat brain neuroactive steroid concentrations and GABAA receptor function after acute stress. Neuroendocrinology. 1996;63:166-72. Medline:9053781 doi:10.1159/000126953
67 Altemus M. Sex differences in depression and anxiety disorders: potential biological determinants. Horm Behav. 2006;50:534-8. Medline:16920114 doi:10.1016/j.yhbeh.2006.06.031
68 Alexander JL, Dennerstein L, Kotz K, Richardson G. Women, anxiety and mood: a review of nomenclature, comorbidity and epidemiology. Expert Rev Neurother. 2007;7 (11 Suppl):S45-S58. Medline:18039068 doi:10.1586/14737175.7.11s.S45
69 Blier P. Norepinephrine and selective norepinephrine reuptake inhibitors in depression and mood disorders: their pivotal roles. J Psychiatry Neurosci. 2001;26 Suppl:S1-2. Medline:11590963
70 Millan MJ. Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application. Pharmacol Ther. 2006;110:135-370. Medline:16522330 doi:10.1016/j.pharmthera.2005.11.006