J. Endocrinol. Invest. *25:* 420-425, 2002

Alprazolam, a benzodiazepine, does not modify the ACTH and cortisol response to hCRH and AVP, but blunts the cortisol response to ACTH in humans

S. Grottoli, B. Maccagno, J. Ramunni, L. Di Vito, R. Giordano, L. Gianotti, S. Destefanis, F. Camanni, E. Ghigo, and E. Arvat

Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Turin, Italy

ABSTRACT. Alprazolam (AL), a benzodiazepine which activates gamma-amino butyrric acid (GABA)-ergic receptors, exerts a clear inhibitory effect on the activity of the hypothalamo-pituitary-adrenal (HPA) axis and is able to markedly reduce the ACTH response to metyrapone-induced inhibition of glucocorticoid feedback. It has been suggested that its inhibitory action could be regulated by CRH or AVP mediated mechanisms. However, the effect of benzodiazepines on the HPA response to CRH or AVP is contradictory. It has been shown that benzodiazepines have specific receptors on the adrenal gland but it is unclear if they mediate biological effects in humans. In order to further clarify the mechanisms underlying the inhibitory effect of benzodiazepine on HPA axis in humans, we studied the effect of AL (0.02 mg/kg po at –90 min) or placebo in 7 healthy young volunteers (7 female, age: 26-34 yr; wt: 50-58 kg, BMI 20-22 kg/m^2) on: 1) the ACTH and cortisol responses to hCRH (2.0 µg/kg iv at 0 min) or AVP (0.17 U/kg im at 0 min); 2) the cortisol, aldosterone and DHEA responses to ACTH 1-24 (0.06 and 250 µg iv at 0 and 60 min, respectively). After placebo, the ACTH and cortisol responses to hCRH (peaks, mean±SE: 29.8± 4.4 pg/ml and 199.3±19.6 µg/l) were similar to those recorded after AVP (31.7±6.5 pg/ml and 164.8±18.0 µg/l); the cortisol response to 0.06 µg ACTH (190.4±11.8 µg/l) was similar to that recorded after hCRH and AVP but lower (p<0.01) than that after 250 µg ACTH (260.6±17.4 µg/l). AL did not modify the ACTH response to both hCRH (42.5±7.1 pg/ml) and AVP (33.3±2.7 pg/ml), which even showed a trend toward increase. AL also failed to significantly modify the cortisol response to both hCRH (156.3±12.7 µg/l) and AVP (119.4±14.5 µg/l), which, on the other hand, showed a trend toward decrease. The cortisol peaks after 0.06 µg ACTH were significantly reduced (p<0.02) by AL pre-treatment (115.0±7.7 µg/l) which, in turn, did not modify the cortisol response to the subsequent ACTH bolus (214.7±16.6 µg/l). The DHEA and aldosterone responses to all the ACTH doses were not significantly modified by AL. In conclusion, these data show that the HPA response to AVP as well as to hCRH is refractory to the inhibitory effect of AL which, in turn, blunts the cortisol response to low ACTH dose. These findings suggest that both CRH- and AVP-mediated mechanisms could underlie the CNS-mediated inhibitory effect of AL on HPA axis; in the meantime, these results suggest that benzodiazepines could also act on adrenal gland by blunting the sensitivity of the fasciculata zone to ACTH.

(J. Endocrinol. Invest. *25:* 420-425, 2002)

INTRODUCTION

Alprazolam (AL) is a benzodiazepine which possesses a marked inhibitory effect on the activity of hypothalamo-pituitary-adrenal (HPA) axis both in animal and in man (1-9). The inhibitory effect of benzodiazepines, particularly of AL, on HPA includes their ability to increase central gamma-amino butyrric acid (GABA)-A activity, which plays

Key-words: Alprazolam, hCRH, AVP, ACTH, cortisol.

Correspondence: Prof. Emanuela Arvat, Divisione di Endocrinologia, Ospedale Molinette, C.so Dogliotti 14, 10126 Torino, Italy.

E-mail: emanuela.arvat@unito.it

Accepted November 26, 2001.

a major role in the neural control of HPA axis (10). In humans, AL significantly reduces spontaneous ACTH and cortisol secretion 24 h urinary cortisol levels (11), the ACTH and cortisol response to stress as well as to AVP, naloxone, an opioid antagonist, and hexarelin, a synthetic GH secretagogue (3, 5, 6-9, 11, 12). More recently, AL has even been shown to markedly inhibit the corticotroph response to metyrapone-induced removal of the negative glucocorticoid feedback (13), suggesting a crucial role of GABA-ergic pathways on the neuroregulation of HPA axis. Animal and human studies suggested a CRH-mediated action for benzodiazepines, in agreement with evidence that GABA has an inhibitory role on CRH-secreting neurons and that AL does not modify the cortisol response to hCRH (4, 14-17). However, in humans the HPA response to hCRH has been reported to be reduced by temazepam, suggesting an AVP-mediated mechanism of action of benzodiazepines (18), in agreement with data *in vitro* and *in vivo* showing that both benzodiazepines and GABA inhibit hypothalamic AVP release (19-20). These data are, however, in disagreement with the evidence in humans showing that AL blunts HPA response to AVP (8).

It has been demonstrated that benzodiazepines have also specific receptor subtypes in animal and human adrenal gland (21) and some data indicate that these receptors are likely to play a major role in the regulation of the adrenal steroidogenesis as well as in the adrenal response to stress in infant rats (22-24). However, the effect of benzodiazepines on the adrenal response to ACTH has never been studied in humans.

Based on the foregoing, we had 2 aims in the present study of healthy young volunteers: 1) to further verify the effects of AL on the ACTH and cortisol response to hCRH or AVP; 2) to study the effects of AL pre-treatment on the adrenal response to low and supramaximal ACTH doses.

MATERIALS AND METHODS

Peptides and drugs

Vials containing 100 µg hCRH (Human CRH) were purchased from Ferring (Kiel, Germany). Vials containing 20 U AVP were purchased from Parke-Davis (Berlin, Germany). Vials containing 250 µg ACTH 1-24 (tetracosactin, Synacthen) were purchased from Novartis Farma (Varese, Italy). Tablets containing 0.5 mg AL (Xanax) were purchased from Pharmacia&Upjohn (Milan, Italy).

Study design

Seven healthy young women (age 26-34 yr; wt 50-58 kg, BMI 20-22 kg/m^2) were studied in their early follicular phase.

The study was approved by the local Ethical Committee of the University of Turin and informed consent was obtained from all subjects.

All subjects underwent the following treatments, in random order and at least 3 days apart, starting at 07:00 h after an overnight fast and 30 min after venous cannulation kept patent by slow infusion of isotonic saline: 1) placebo (po at –90 min) + hCRH (2.0 µg/kg iv at 0 min); 2) placebo + AVP (0.17 U/kg im at 0 min); 3) AL (1.0 mg po at -90 min, approximatively 0.02 mg/kg in subjects of 54.5±1.8 kg) + hCRH; 4) AL + AVP; 5) placebo + ACTH (ACTH 1-24, 0.06 and 250 µg iv at 0 and 60 min, respectively); 6) AL + ACTH.

In testing sessions 1-4, blood samples were taken at -90 and 0 min and then every 15 min up to 120 min after iv administration of hCRH or AVP. In testing sessions 5 and 6, blood samples were taken at –90 and 0 min and then at +15, +30, +60, +90 and +120 min.

All samples from an individual subject were analyzed together for ACTH and cortisol in testing sessions 1-4 or for cortisol, aldosterone and DHEA in testing sessions 5 and 6.

Plasma ACTH levels (pg/ml) were measured in duplicate by IRMA (Allegro HS-ACTH, Nicholls Institute Diagnostic, San Juan Capistrano, U.S.A.). The sensitivity of the assay was 1.0 pg/ml. The inter- and intra-assay coefficients of variation ranged from 2.4 to 8.5% and from 3.9 to 9.9%, respectively.

Serum cortisol levels (µg/l) were measured in duplicate by RIA (CORT-CTK 125, IRMA, SORIN, Saluggia, Italy). The sensitivity of the assay was 0.2 µg/l. The inter- and intra-assay coefficients of variation ranged from 4.3 to 14.6% and from 5.7 to 9.9%, respectively.

Serum DHEA levels µg/l were measured in duplicate by RIA (Active DHEA, Diagnostic Systems Laboratories, Webster, U.S.A.). The sensitivity of the assay was 0.02 ng/ml. The inter- and intra-assay coefficients of variations ranged from 11.2 to 13.7 % and from 5.2 to 6.2%, respectively.

Serum aldosterone levels (pg/ml) were measured in duplicate by RIA (Aldosterone, MAIA, RIA Biochem Immunosystems Company, Casalecchio di Reno, Bologna, Italy). The sensitivity of the assay was 6 pg/ml. The inter- and intra-assay coefficients of variations ranged from 11.9 to 14.1% and from 5.8 to 12.5%, respectively.

The hormonal responses are expressed either as absolute values or as AUC, calculated by trapezoidal integration.

The statistical analysis was carried out using a non-parametric ANOVA (Friedman test) and then with Wilcoxon test. Results are expressed as mean±SE.

RESULTS

Basal hormonal levels in various testing sessions without AL pre-treatment were similar.

After placebo pretreatment, the ACTH and cortisol responses to hCRH (peaks, mean±SE: 29.8±4.4 *vs* 17.4±3.1 pg/ml and 199.3±19.6 *vs* 114.8±12.3 µg/l, $p<0.05$) were similar to those recorded after AVP (31.7±6.5 *vs* 14.9±3.5 pg/ml and 164.8±18.0 *vs* 107.2±8.5 µg/l, $p<0.05$) (Fig. 1).

The cortisol response to 0.06 µg ACTH (190.4±11.8 *vs* 115.6±12.9 µg/l, $p<0.05$) was similar to that

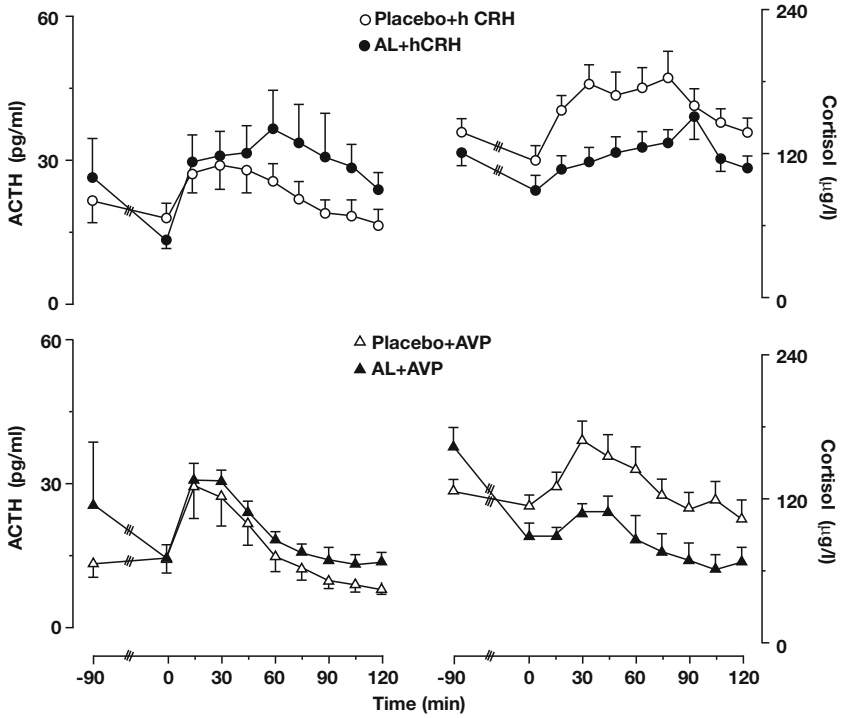

Fig. 1 - *Mean±SE ACTH and cortisol responses to hCRH or AVP preceded by placebo or alprazolam (AL) in healthy subjects.*

recorded after hCRH and AVP but lower ($p<0.01$) than after 250 µg (260.6±17.4 *vs* 136.1±12.3 µg/l, $p<0.05$) (Fig. 2).

ACTH administration also induced a significant and dose-related ($p<0.02$) DHEA increase (0.06 µg: 12.6±1.0 *vs* 8.1±0.5 µg/l, $p<0.05$; 250 µg: 19.1±2.5 *vs* 8.2±1.0 µg/l, $p<0.05$), while the aldosterone response to 0.06 µg ACTH was lower than that to 250 µg, though this, difference did not attain statistical significance (0.06 µg: 214.1±17.0 *vs* 146.3±15.4 pg/ml, $p<0.05$; 250 µg: 266.4±30.0 *vs* 131.5±11.0 pg/ml, $p<0.05$).

AL pre-treatment lowered ($p<0.05$) basal cortisol and DHEA but not ACTH and aldosterone levels.

AL pre-treatment did not significantly modify the ACTH response to both hCRH (42.5±7.1 *vs* 12.7±1.7 pg/ml) and AVP (33.3±2.7 *vs* 14.4±2.8 pg/ml), which showed even a non-significant trend toward increase.

AL did not significantly modify the cortisol response to hCRH (156.3±12.7 *vs* 90.5±12.7 µg/l) or AVP (119.4±14.5 *vs* 88.1±8.0 µg/l) which, however, showed a trend toward decrease (Fig. 1).

By evaluating ACTH/cortisol ratios during hCRH and AVP sessions both alone and after AL pre-treatment, an increase in ACTH/cortisol ratios was recorded when hCRH or AVP had been preceded by AL ($p<0.05$).

The cortisol response to 0.06 µg ACTH was significantly reduced ($p<0.02$) by AL pre-treatment (115.0±7.7 *vs* 78.4±4.5 µg/l) which, in turn, did not modify the cortisol response to the subsequent ACTH bolus (214.7±16.6 *vs* 84.9±4.5 µg/l) (Fig. 2).

AL did not significantly modify DHEA and aldosterone responses to both 0.06 and 250 µg ACTH

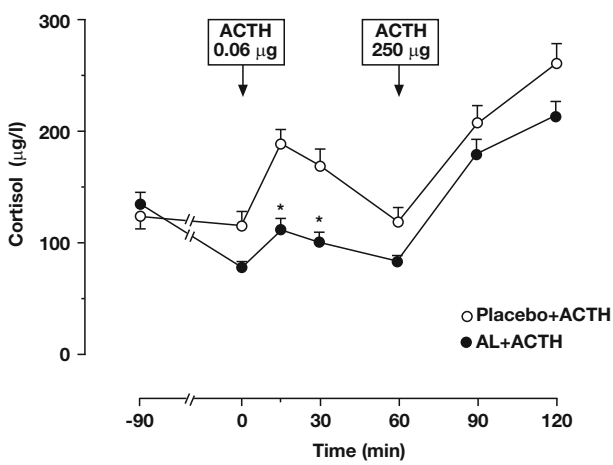

Fig. 2 - *Mean±SE cortisol responses to ACTH preceded by placebo or alprazolam (AL) in healthy subjects. *p<0.02.*

dose (DHEA, 0.06 µg: 8.8±1.0 *vs* 5.9±0.6 µg/l; 250 µg: 15.9±1.6 *vs* 6.6±0.9 µg/l; aldosterone, 0.06 µg: 240.1±54.5 *vs* 195.9±40.0 pg/ml; 250 µg: 393.0±96.0 *vs* 166.1±36.9 pg/ml).

Side-effects

Transient facial flushing was recorded in all subjects after hCRH administration. Mild nausea and vasoconstriction were recorded in all 3 subjects, respectively, after AVP administration. No side-effect was recorded after ACTH 1-24 administration. After AL administration mild sleepiness was recorded in 4 subjects. No medication was required and there was no need to interrupt the tests.

DISCUSSION

The results of the present study show that the HPA response to AVP as well as that to hCRH are refractory to the inhibitory effect of AL. The stimulatory effect of hCRH or AVP on HPA after AL pretreatment is characterized by an increase in ACTH/cortisol ratios. Moreover, AL pretreatment blunts the cortisol response to a low but not to the supramaximal ACTH dose. On the contrary, the response of other adrenal steroids, namely DHEA and aldosterone, to both low and supramaximal ACTH doses is not modified by benzodiazepine pre-treatment.

The strong central nervous system (CNS) mediated inhibitory effect of AL, a benzodiazepine, on the activity of HPA axis has clearly been demonstrated both in animals and in humans (1-9, 12, 14, 15, 17). The central mechanisms underlying the inhibitory effect of benzodiazepines, particularly of AL, on HPA activity involve the ability of benzodiazepines to increase central GABA-A activity (10), which plays a major role in the neuroregulation of HPA axis, probably modulating the activity of CRH- and or AVP-secreting neurons (4, 7-9, 14, 15, 17, 19, 20). On the other hand, it has also been suggested that AL may inhibit noradrenergic activity which, in turn, plays stimulatory influence on corticotroph function (5, 25-27).

In agreement with a CRH-mediated action for benzodiazepines, AL has been shown to inhibit CRH release in the *locus coeruleus*, in the paraventricular nucleus and the serotonin-induced CRH release from rat hypothalamus (4, 14, 17), while it does not modify either spontaneous or CRH-stimulated ACTH release from isolated rat pituitary cells (4, 15). In humans, AL has been reported to be unable to modify the HPA response to hCRH (9) which, however, was found blunted by temazepam (18). Our present findings confirm the inability of AL to inhibit the hCRH-induced ACTH response which, in the present study, even showed a trend toward increase; this was coupled with the opposite trend of cortisol response. These findings fit well with the hypothesis that, at least partially, the inhibitory effect of benzodiazepine on HPA axis involves negative influence on CRH-secreting neurons.

Our present results show that also the AVP-induced corticotroph response is not reduced by AL pretreatment. Similarly to what observed after hCRH, the ACTH response to AVP after AL even showed a trend toward increase; once again this was coupled with the opposite trend of cortisol release. Our results do not agree with previous ones showing a slight but significant reduction of AVP-induced ACTH and cortisol increase after AL (8). Different study protocols and/or AVP or AL doses could explain this discrepancy. Indeed, our present findings fit well with data *in vitro* and *in vivo* indicating that benzodiazepines, as well as GABA, also play an inhibitory role on hypothalamic AVP release (19, 20). Thus, our present results suggest that benzodiazepines exert their inhibitory effect on the HPA activity via concomitant actions on both CRH- and AVP-secreting neurons within the hypothalamus or at a suprahypothalamic level within the hippocampus, where GABA-A receptors have widely been identified (27). This hypothesis could also explain the marked inhibitory effect of AL on the corticotroph responsiveness to metyrapone (13), which probably acts concomitantly via central stimulation of CRH and AVP release (28), and on HPA response to canrenoate (29), an antagonist of the mineralocorticoid receptors.

It has been demonstrated that benzodiazepines have specific receptor subtypes also in animal and human adrenal gland (21). In animals these receptors play a role in the control of glucocorticoid synthesis and release and there is evidence that benzodiazepines influence the maturation of the glucocorticoid responsiveness to stress in infant rats (22-24). While the adrenal response to ACTH has been reported to be reduced or unchanged by benzodiazepines in animals (21), the effect of these substances on the adrenal response to ACTH in humans has never been studied. To address this point we verified the effect of AL on cortisol, DHEA and aldosterone responses to a very low and the supramaximal ACTH doses, administered as consecutive boluses. Our findings showed that AL clearly blunted the cortisol response to the low but not to the supramaximal ACTH dose. Notice that the cortisol response to the low ACTH dose preceded by placebo was similar to

that after hCRH or AVP. Thus, these findings point toward direct inhibitory effect of AL on the adrenal gland and agree with evidence that, after AL pretreatment, the HPA response to hCRH or AVP is characterized by an increase in ACTH/cortisol ratios. Therefore, benzodiazepines probably act on the adrenal gland by reducing the sensitivity of the fasciculata zone to ACTH; in fact, both DHEA and aldosterone responses to ACTH were unaffected by AL pre-treatment.

In conclusion, our study indicates that both CRH- and AVP-mediated mechanisms are likely to underlie the central inhibitory effect of AL on HPA axis and points toward the hypothesis that benzodiazepines also act on the adrenal gland by blunting the sensitivity to ACTH in adrenal fasciculata zone. From a clinical point of view, these results also point toward potential contraindication of AL administration in patients with suspected hypoadrenalism.

ACKNOWLEDGEMENTS

This study was supported by MURST (n. 9906153187), University of Turin (grant 1998) and SMEM Foundation. The Authors wish to thank Dr. M. Maccario for his support of the study and A. Bertagna and A. Barberis for their skillful technical assistance.

REFERENCES

1. Charney D.S., Breier A., Jatlow P.I., Heninger G.R. Behavioural, biochemical and blood pressure responses to alprazolam in healthy subjects: interactions with yohimbine. Psychopharmacology (Berl.) 1986, *88:* 133-140.
2. Calogero A.E, Galactic W.T., Chrousos G.P., Gold P.W. Interaction between GABAergic neurotransmission and rat hypothalamic corticotropin-releasing hormone secretion in vitro. Brain Res. 1988, *463:* 28-36.
3. Risby E.D., Hsiao J.K., Golden R.N., Potter W.Z. Intravenous alprazolam challenge in normal subjects. Biochemical, cardiovascular, and behavioral effects. Psychopharmacology (Berl.) 1989, *99:* 508-514.
4. Kalogeras K.T., Calogero A.E., Kuribayiashi T., *et al. In vitro* and *in vivo* effects of the triazolobenzodiazepine alprazolam on hypothalamo-pituitary-adrenal function: pharmacological and clinical implications. J. Clin. Endocrinol. Metab. 1990, *70:* 1462-1471.
5. Zemishlany Z., McQueeney R., Gabriel S.M., Davidson M. Neuroendocrine and monoaminergic responses to acute administration of alprazolam in normal subjects. Neuropsychobiology 1990, *23:* 124-128.
6. Breier A., Davis O., Buchanan R., *et al*. Effects of alprazolam on pituitary-adrenal and catecholaminergic responses to metabolic stress in humans. Biol. Psychiatry 1992, *32:* 880-890.
7. Torpy D.J., Grice J.E., Hockings G.I., Walters M.M., Crosbie G.V., Jackson R.V. Alprazolam blocks the naloxone-stimulated hypothalamo-pituitary-adrenal axis in man. J. Clin. Endocrinol. Metab. 1993, *76:* 388-392.
8. Torpy D.J., Grice I.E., Hockings G.I., Walters M.M., Crosbie G.V., Jackson R.V. Alprazolam attenuates vasopressin-stimulated adrenocorticotropin and cortisol release: evidence for synergy between vasopressin and corticotropin-releasing hormone in humans. J. Clin. Endocrinol. Metab. 1994, *79:* 140-144.
9. Roher T., von Richthofen V., Schulz C., Beyer J., Lehnert H. The stress, but not corticotropin-releasing hormone-induced activation of the pituitary-adrenal axis in man is blocked by alprazolam. Horm. Metab. Res. 1994, *26:* 200-206.
10. Prichett D.B., Sontheimer H., Shivers B., et al. Importance of a novel GABA A receptor subunit for benzodiazepine pharmacology. Nature 1989, *338:* 582-585.
11. Lopez A.L, Katol R.G, Noyes R. Reduction in urinary free cortisol during benzodiazepine treatment of panic disorder. Psychoneuroendocrinology 1990, *15:* 23-28.
12. Arvat E., Maccagno B., Ramunni J., *et al*. Effects of dexametasone and alprazolam, a benzodiazepine, on the stimulatory effect of Hexarelin, a synthetic GHRP, on ACTH, cortisol and GH secretion in humans. Neuroendocrinology 1998, *67:* 310-316.
13. Arvat E., Maccagno B., Ramunni J., *et al*. The inhibitory effect of alprazolam, a benzodiazepine, overrides the stimulatory effect of metyrapone-induced lack of negative cortisol feedback on corticotroph secretion in humans. J. Clin. Endocrinol. Metab. 1999, *84:* 2611-2615.
14. Cedarbaum J.M., Aghajanian G.K. Catecholamine receptors on locus coeruleus neurones: pharmacological characterization. Eur. J. Pharmacol. 1977, 44: 375-385.
15. Hashimoto K., Yunoki S., Takahara J., Ofuji T. ACTH release in pituitary cell cultures. Effects of neurogenic peptides and neurotransmitter substances on ACTH release induced by hypothalamic corticotropin releasing factor (CRF). Endocrinol. Jpn. 1979, *26:* 103-109.
16. Gaillard R.C., Al-Damluji S. Stress and the pituitary-adrenal axis. Baillieres Clin. Endocrinol. Metab. 1987, *2:* 319-349.
17. Owens M.J., Bissette G., Neneroff C.B. Acute effects of alprazolam and adinazolam on the concentrations of corticotropin – releasing factor in the rat brain. Synapse 1989, *4:* 196-202.
18. Korbonits M., Trainer P.J., Edwards R., Besser J.M., Grossman A.B. Benzodiazepines attenuate the pituitary-adrenal responses to corticotrophin-releasing hormone in healthy volunteers but not in patients with Cushing's syndrome. Clin. Endocrinol. 1995, *43:* 29-35.
19. Wible J.H., Zerbe R.L., Di Micco J.A. Benzodiazepine receptor modulate circulating plasma vasopressin concentration. Brain Res. 1985, *359:* 368-370.
20. Hillhouse E.W., Milton N.G.N. Effect of noradrenaline and gamma-aminobutyrric acid on the secretion of corticotrophin-releasing factor-41 and arginine vasopressin from rat hypothalamus *in vitro*. J. Endocrinol. 1989, *122:* 719-723.

21. Papadopolous V. Pheripheral-type benzodiazepine/diazepam binding inhibitor receptor: biological role in steroidogenic cell function. Endocr. Rev. 1993, *14:* 222-240.
22. Yanagibashi K., Ohno Y., Nakamichim N., *et al*. Peripheral-type benzodiazepine receptors are involved in the regulation of cholesterol side chain cleavage in adrenocortical mitochondrial. J. Biochem. 1989, *106:* 1026-1029.
23. Van Oers H.J.J., de Kloet E.R., Li C., Levine S. The ontogeny of glucocorticoid negative feedback: influence of maternal deprivation. Endocrinology 1998, *139:* 2838-2846.
24. Zilz A., Hua L., Castello R., Papadopoulos V., Widmaier E.P. Developmental expression of the peripheral-type benzodiazepine receptor and the advent of steroidogenesis in rat adrenal glands. Endocrinology 1999, *140:* 859-864.
25. Eriksson E., Carlsson M., Nilsson C., Soderpalm B. Does alprazolam, in contrast to diazepam, activate alpha-2-adrenoceptors involved in the regulation of rat growth hormone secretion? Life Sci. 1986, *39:* 1491-1498.
26. Plotsky P.M., Cunningham E.T., Widmaier E.P. Catecholaminergic modulation of corticotropin-releasing factor and adrenocorticotropin secretion. Endocr. Rev. 1989, *10:* 437-445.
27. De Kloet E.R., Vreugdehil E., Oitzl M.S., Joels M. Brain corticosteroid receptor balance in health and disease. Endocr. Rev. 1998, *19:* 269-301.
28. Conte-Devolx B., Guillaume V., Boudouresque F., *et al*. Effects of metyrapone infusion on corticotropin-releasing factor and arginine vasopressin secretion into the hypophysial portal blood of conscious, unrestrained rams. Acta Endocrinol. 1992, *127:* 435-440.
29. Arvat E., Maccagno B., Giordano R., *et al*. Mineralocorticoid receptor blockade by canrenoate increases both spontaneous and stimulated adrenal function in humans. J. Clin. Endocrinol. Metab. 2001, *86:* 3176-3181.