Response of the hypothalamic-pituitary-adrenal axis to small dose arginine-vasopressin and daily urinary free cortisol before and after alprazolam pre-treatment differs in obesity

V. Vicennati, L. Ceroni, L. Gagliardi, U. Pagotto, A. Gamberini, S. Genghini, and R. Pasquali
Division of Endocrinology, Department of Internal Medicine, S. Orsola-Malpighi Hospital, University of Bologna, Italy

ABSTRACT. Objective: Arginine vasopressin (AVP) has a central role in the response of the hypothalamic-pituitary-adrenal (HPA) axis to stress conditions. A low dose of AVP has been shown to have a modest, but significant effect on ACTH response in normal weight subjects. The aim of this study was to test the response of the HPA axis in obese subjects in order to assess eventual primary neuroendocrine alterations, previously demonstrated by using AVP combined with corticotropin releasing hormone (CRH). In addition, given its central inhibitory action on the HPA axis, we investigated whether the suppressive capacity of alprazolam (APZ) pretreatment on the hormone response to low-dose AVP challenge and daily urinary free cortisol (UFC) excretion rate may be altered in the presence of obesity. Design: Fifteen overweight or obese women and eight normal-weight controls randomly underwent two low-dose AVP tests (0.3 Ul iv bolus), one without (AVP test) and the other preceded by APZ administration (0.5 mg at midnight and 0.5 mg 90 min before the test in the morning at 08:30 h) (APZ/AVP test). Blood samples for ACTH and cortisol assay were obtained at baseline and throughout each test. The day before each test, 24h-UFC/creatinine was also mea-sured. Results: Basal ACTH levels were similar in the two groups, whereas cortisol concentrations were significantly lower in the overweight/obese group. Overweight/obese women had higher ACTH and cortisol responses to the AVP tests and significantly greater hormone inhibition after APZ than controls. In both groups, APZ-induced Δ-peak cortisol values before and after APZ pre-treatment were significantly correlated. Body fat distribution had no effect on the HPA axis response to AVP either before or after APZ. Moreover, APZ decreased 24h-UFC/creatinine values unsignificantly in controls and by approximatively 50% in the overweight/obese subjects. These changes were unrelated to the cortisol response to the AVP test before and after APZ pretreatment. On the other hand, percent changes of 24h-UFC/creatinine after APZ were negatively related to the body mass index (BMI) but positively with waist circumference values, which indicates that the abdominal obesity phenotype may counteract the 24 h-UFC/creatinine that would be expected on the basis of BMI values. Conclusions: Our data further support the concept that in women obesity may represent a condition of hyperresponsiveness or hypersensitivity of the HPA axis to neuroendocrine stimuli, which appear to be independent of feedback control. In addition, the data on the inhibiting capacity of APZ on UFC excretion confirm that the alterations of the HPA axis in obesity is particularly evident in the abdominal phenotype. (J. Endocrinol. Invest. 27: 541-547, 2004) ©2004, Editrice Kurtis

INTRODUCTION
The major ACTH secretagogues in humans are corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) (1), and their combined administration has synergistic effects on ACTH release in vivo (2, 3). This synergy is important in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis, as

Key-words: Obesity, HPA axis, arginine vasopressin, alprazolam, urine free cortisol.
Correspondence: Prof. R. Pasquali, Divisione di Endocrinologia, Dipartimento di Medicina Interna, S. Orsola-Malpighi Hospital, Via Massarenti 9, 40138 Bologna, Italy.
E-mail: renato.pasquali@unibo.it
Accepted March 16, 2004.
CRH and AVP release can be differentially regulated, particularly after qualitatively different stressors (2-5). The mechanisms may depend on different corticotrope populations, some of which require both secretagogues for ACTH release (6-7) or on coupling between the corticotrope intracellular signaling mechanisms for the secretagogues (8).

In subjects with the abdominal obesity phenotype, ACTH and cortisol response to both CRH alone (9) or combined with AVP (10, 11) is higher than that observed in their peripheral obesity counterparts and in non-obese individuals. Although the precise mechanisms of these abnormalities are not completely elucidated, there is evidence that they may reflect a central dysregulation of the HPA axis. In fact, several studies performed in humans (11-13) support the concept that altered HPA axis activity in obesity may be favoured by a disorganisation of the catecholaminergic regulation in the central nervous system, particularly during prolonged stress challenges, as suggested by previous data obtained in animal studies (14, 15). Alprazolam (APZ) is a triazolobenzodiazepine mostly used for the treatment of endogenous depression and panic disorder (16) which inhibits the HPA axis via the activation of central γ-aminobutyric acid (GABA) system and through the direct inhibition of hypothalamic CRH release (14, 17, 18). Studies in humans have shown that APZ significantly reduces daily urinary free cortisol (UFC) excretion rates in both psychiatric patients (19) and healthy subjects. Moreover APZ reduces ACTH and cortisol response to acute stress challenges (20) and AVP (21), and markedly inhibits the ACTH response to metyrapone (22), which suggests an important role of the GABAergic system in the neuroregulation of the HPA axis. In addition, there are data demonstrating that APZ may inhibit central noradrenergic activity (23), which may further explain a central inhibitory effect on the HPA axis.

In this study we tested the hypothesis that if a higher than normal ACTH and cortisol response to low-dose AVP is found in subjects with obesity, this indirectly reflects primary alterations of central neuroendocrine mechanisms regulating the HPA axis. By considering the central role of AVP in the response of the HPA axis to stress conditions, we therefore investigated the hormone response to acute low-dose (0.3 UI) AVP stimulation in normal-weight women and in overweight/obese women with a wide range of body fat distribution. This dose has been shown to have only a modest effect on ACTH secretion when administered alone, whereas the same dose practically doubled the ACTH response when administered together with 100 μg of human CRH (24). In addition, given its central inhibitory action on the HPA axis, we investigated whether the suppressive capacity of APZ pre-treatment on the hormone response to low-dose AVP challenge and daily UFC excretion rate may be altered in the presence of obesity.

MATERIALS AND METHODS

Subjects

This study included 15 overweight and obese subjects [body mass index (BMI) ranged from 27 to 40 kg/m²] and 8 normal-weight control women. All had regular menses and were regularly ovulating. Based on clinical history, physical examination and laboratory data, none of them had thyroid diseases, Cushing's syndrome, hyperandrogenism, hypertension or cardiovascular, renal, hepatic or systemic diseases, nor were they taking any type of medications, drugs or dieting. Diabetes was excluded by performing an oral glucose tolerance test, according to the American Diabetes Association criteria (25). Moreover, none of them had depression (see below for exclusion criteria), was a habitual smoker or consumed more than 30 g/day of alcohol. Finally, none of the controls had a history of having been overweight or obese and none of them were smokers. In the days before and during the study, all women were invited to follow their usual diet, provided carbohydrate ingestion was maintained within 250-300 g/day. All women gave informed and written consent to the study, which had been approved by the local Ethics Committee.

Psychological evaluation

The presence of depressive traits was investigated by means of two different questionnaires, the clinical depression scale (CDQ) (26) and the center for Epidemiologic Studies of Depression (CES-D) (27), both in the Italian version, as previously reported (11).

Anthropometry

Height was measured without shoes to the nearest 0.5 cm, and body weight was taken without clothes. The waist (W) and hip (H) circumferences were also measured, with the subjects standing, using a 1-cm-wide metal measuring-tape, and the waist-to-hip ratio (WHR) was calculated. Waist and hip circumferences were measured according to the World Health Organization (WHO) recommendations (28). A cut-off value lower or higher than 88 cm was chosen as of the peripheral or abdominal obesity phenotype.

Protocol

Tests were performed in the morning (08:00 h) and in the follicular phase of the menstrual cycle, which is never more than 10 days after the start of the previous menstrual cycle. Each subject underwent two AVP tests, one without (AVP test) and the other preceded by APZ administration (APZ/AVP test). The sequence of each pair of tests in each subject was randomized. Therefore 8 overweight/obese and 4 control women had the simple AVP test first, whereas 7 obese and 4 controls began with the APZ/AVP test. An interval of 3-4 days occurred between each test. Each test was performed as follows. An iv catheter for blood collection was placed in a forearm vein of one arm and the vein was kept patent with sodium chloride (NaCl) (0.9%) infusion for at least 30 min. Two blood samples for cortisol and ACTH determination were collected at baseline at 15 min intervals and 2, 5, 10, 15, 30 min after an iv bolus of AVP (Pitressin, Parke Davis, Berlin, Germany).