Impaired beta-endorphin response to human corticotropin-releasing hormone in obese children

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Abstract. In order to evaluate the secretion of beta-endorphin in obese children and adolescents, we measured plasma beta-endorphin, ACTH and cortisol levels before and following administration of CRH (1 μg/kg). Fourteen normal weight and 22 obese subjects (weight excess ranging from 30 to 98%) were studied. Plasma hormone levels were measured by radioimmunoassay directly in plasma (cortisol, ACTH) and after silicic acid extraction and Sephadex G-75 column chromatography (beta-endorphin). Basal beta-endorphin levels in obese children were significantly higher than in controls (14.7 ± 1.8 vs 6.0 ± 0.6 pmol/l; mean ± SEM). No differences were found in basal ACTH and cortisol levels. CRH administration significantly increased beta-endorphin, ACTH and cortisol levels in normal subjects and ACTH and cortisol levels in obese subjects. Plasma beta-endorphin levels in obese children and adolescents did not show any significant increment. These data confirm the higher than normal beta-endorphin plasma levels in obese subjects in childhood and demonstrate that CRH is unable to increase beta-endorphin levels, suggesting an impairment of the hypothalamo-pituitary control mechanisms or an extra-anterior pituitary source.

Beta-endorphin (B-EP), an endogenous opioid peptide, stimulates food intake when centrally injected in rats (Grandison & Guidotti 1977) and in sheep (Baile et al. 1981). Genetically obese rats shows pituitary concentrations of B-EP higher than those of control rats, suggesting a possible role of this peptide in obesity, although this finding could be a consequence rather than a cause of this condition (Margules et al. 1978; Rossier et al. 1979). Moreover, recent reports show that obese subjects have plasma B-EP levels 3-fold higher than normal healthy subjects, supporting a hypersecretion of the peptide in this pathological condition (Givens et al. 1980; Aleem & McIntosh 1984; Fullerton et al. 1986). This endocrine pattern is evident also in obese children and adolescents (Genazzani et al. 1986), suggesting that in obesity the mechanisms regulating B-EP secretion are impaired irrespective of the patient’s age (Facchinetti et al. 1987). In normal conditions B-EP secretion is regulated by the same mechanisms controlling ACTH and beta-lipotropin (B-LPH) (Olson et al. 1984). CRH increases circulating B-EP concomitantly with ACTH and B-LPH in human beings (McLoughlin et al. 1984). However, in obese subjects, plasma B-EP levels do not have the same circadian changes as ACTH or B-LPH and are resistant to dexamethasone suppression (Facchinetti et al. 1987) suggesting a selective impairment of the mechanisms regulating B-EP secretion or a possible extra-pituitary source. Also, our previous studies showed an impaired B-EP response to clonidine, an α₂ adrenergic agonist, and to fluoxetine, a serotonin re-uptake blocker (Bernasconi et al. 1987).

In order to evaluate B-EP secretion in obese children and adolescents, we measured in the present study the plasma B-EP, ACTH and cortisol levels after CRH administration.
Subjects and Methods

After informed parental consent, 36 subjects entered the study and were subdivided into two groups according to body weight. The control group consisted of 14 healthy, normal weight (± 10% of ideal body weight) children and adolescents (age range from 8.2 to 11.6 years). The patient group was composed of 22 obese (body weight excess range from 30 to 98%; mean ± SD 55 ± 10%) children and adolescents (age range from 8.1 to 15.8 years). Personal history as well as clinical and previously laboratory evaluations were consistent with the diagnosis of simple exogenous obesity.

After an overnight fast, the subjects attended the endocrinological ward at 08.00 h on separate days. An antecubital vein was cannulated and kept patent using saline. Two basal blood samples were collected 15 min apart and had Trasylol (1000 KIU/ml) and heparin added. Then human CRH (1 µg/kg iv, Nova Biochem, Switzerland) was injected as a bolus dose. Further blood samples were collected 15, 30, 45, 60 and 120 min later and immediately centrifuged and stored at -20°C until assayed.

Patients and control were also studied following a placebo administration (0.9% saline injection, iv) with samples collected as following CRH.

No adverse reactions were observed after administration of any of the drugs and the blood pressure did not change significantly.

B-EP was measured in plasma (3–4 ml) and extracted with silicic acid, as previously described (Genazzani et al. 1983). The extracts were vacuum dried at 37°C, reconstituted in 0.1 mol/l acetic acid and 0.01% BSA, and gel-filtered on Sephadex G-75 columns (45 × 1.5 cm). A 16-ml fraction was collected, corresponding to the elution volume of B-EP, as determined by a detailed evaluation of B-EP-like immunoreactivity in 2-ml fractions from a large pool of plasma extract and also by the elution pattern of iodinated B-EP (Kav = 0.82). The final recovery was 70.1 ± 5% (mean ± SEM). The fractions were freeze-dried and redissolved in 0.04 mol/l phosphate buffer and 0.1% BSA for the RIA (double-antibody method). Each sample was assayed in duplicate. Synthetic B-EP (Organon, Oss, The Netherlands) was used for iodination and standards, and antihuman B-EP serum (C-terminal; Kindly supplied by Prof C. H. Li, San Francisco, CA) for the B-EP RIA. The sensitivity of the RIA was 1.1 fmol. The RIA inter- and intra-assay coefficients of variation were 7.7 and 4.5%, respectively. All samples from each individual subject were extracted and assayed at the same time.

The cortisol and ACTH RIA were performed directly in plasma without any extraction, using commercial kits (Medgenix, Fleurus, Belgium). The assay sensitivity for ACTH was 0.012 ng. The RIA inter- and intra-assay coefficients of variation were 5.9 and 1%, respectively. The sensitivity for cortisol was 18 nmol/l and the inter- and intra-assay CV was 6.2 and 0.7%, respectively.

Statistical analysis of the results was performed using analysis of variance.

Results

Whereas the plasma B-EP levels (mean ± SEM) were significantly higher (14.7 ± 1.8 vs 6.0 ± 0.6 pmol/l, P < 0.01) in obese subjects than in controls, no differences were found for basal ACTH and cortisol levels between the two groups (28.9 ± 4.3 vs 26.7 ± 1.9 ng/l for ACTH and 286.9 ± 30.3 vs 311 ± 66.2 nmol/l for cortisol; mean ± SEM).

In normal weight subjects, CRH administration significantly increased plasma B-EP with peak values at 30 min (P < 0.01 at 15, 30 and 45 min vs basal values, Fig. 1a). On the other hand, in obese patients, CRH did not induce any B-EP response (Fig. 1a).

Moreover, CRH significantly increased plasma ACTH and cortisol levels both in obese patients and in controls (Fig. 1b,c; P < 0.01 at 15, 30, 45 and 60 min vs basal values). Placebo did not induce any significant change of plasma B-EP, ACTH and cortisol levels.

Discussion

In normal subjects, the secretion of pro-opiomelanocortin-related peptides is stimulated by CRH (McLoughlin et al. 1984) and our data indicate that, as previously reported in adults (Grossman et al. 1982), CRH administration significantly increases plasma B-EP, ACTH and cortisol levels in normal children and adolescents. According to recent reports (Genazzani et al. 1986), basal B-EP levels in obese adolescent were higher than in normal controls, confirming that hyperendorphinemia characterizes obesity in childhood. Moreover, these data show that in obese children and adolescents, CRH administration significantly enhances plasma ACTH and cortisol levels, but is unable significantly to increase circulating B-EP levels. Recently, Kopelman et al. (1988) showed an impaired response of plasma cortisol, but not of plasma ACTH, to CRH in obese adult patients. The different results may be explained by the magnitude of body weight excess, which was lower in our patients in comparison to that reported by Kopelman et al. Indeed, the magnitude of body weight...
weight excess is inversely related to the hormonal responses to the dexamethasone test in obese subjects (Facchinetti et al. 1988). The B-EP unresponsiveness to CRH is in agreement with some of our preliminary observations, which showed an impaired response of B-EP to adrenergic, serotonergic, and opioidergic stimuli, suggesting an alteration of these mechanisms in obese children (Bernasconi et al. 1987; Baranowska et al. 1987). Moreover, the absence of any secretory response of B-EP to CRH may also be referred to an ultra-short loop negative feedback, because elevated plasma B-EP inhibits the pituitary response of B-EP to CRH. Finally, in obese subjects, a receptor or postreceptor alteration may also explain the impaired response of B-EP to CRH. Therefore, the evidence that B-EP secretion in obese children and adolescents is not stimulated by CRH, as it is in control subjects, and the dissociation of cortisol, ACTH, and B-EP response reinforce the recent hypothesis of an independent source of this hormone which could be the cause of the hyperendorphinemic state (Facchinetti et al. 1987). The presence of B-EP in different organs, such as the small intestine (Orwoll & Kendall 1980), pancreas (Bruni et al. 1979), gonads (Tsong et al. 1982), has already been demonstrated. Moreover, in experimental animals (Przewlocki et al. 1979) and in fetal pituitaries (Gibbs et al. 1983) previous studies showed that B-EP originates also from the intermediate lobe, probably as the end-product of pro-opiomelanocortin metabolism and that this B-EP secretion is unsensitive to CRH. The extra-anterior pituitary source of hyperendorphinemia in obese subjects is also supported by the unsuppressibility of plasma B-EP by dexamethasone demonstrated in obese children (Facchinetti et al. 1987). However, these data cannot be conclusive for showing an extra-pituitary source of B-EP in obese subjects and the impairment of control regulating mechanisms has to be considered. Indeed, there are several reports showing abnormalities of central neurons in their control of various pituitary secretions (growth hormone, prolactin) (Kopelman et al. 1979; Kopelman & Noonan 1986) in obese subjects.

In conclusion, our data confirm that in obese children and adolescents plasma B-EP levels are higher than in normal weight subjects and demonstrate that CRH is unable to increase B-EP levels.
References


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