ORIGINAL ARTICLE

Plasma corticotropin releasing hormone during the feeling of induced emotions

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Abstract

OBJECTIVES: Central neuropeptides modulate behaviour. Plasma levels of neuropeptides may reflect central levels due to specific brain-to-blood transport systems. We purposed to show the modulation of plasma corticotropin releasing hormone (CRH) levels in relation to induced emotions.

DESIGN: Three groups were defined. For experimental groups A and B, an emotionally significant movie fragment was projected for 20 min, while no film projection occurred in group C. Peripheral venous blood samples were collected before, 10 and 60 min after the film or at 0 and 30 min for group C. Total CRH was measured in plasma. Personality was evaluated by the Minnesota Multiphasic Personality Inventory (MMPI).

RESULTS: Plasma CRH levels did not change in the condition with no movie projection – group C – 346±198 vs. 327±143 pg/mL. Plasma CRH levels dramatically increased with the projection of a dramatic movie – group A – 394±147 vs. 791±636 vs. 803±771 pg/mL, $p<0.05$. Plasma CRH increased less markedly in the condition with the projection of a comic movie – group B – 364±138 vs. 486±260 vs. 483±228 pg/mL, $p<0.05$ for differences between samples 1 and 3. Baseline plasma CRH was significantly and independently related to the neurotic triad and psychotic dyad – partial r=0.328 and 0.267, respectively, $p<0.05$.

CONCLUSIONS: We conclude that plasma CRH levels increase with experimental emotion induction and that baseline levels are significantly related to behavioural traits. Plasma levels of neuropeptides may reflect central levels and may be useful in clinical medicine and in the study of behavioural disorders.

INTRODUCTION

Neural control of the pituitary gland was first postulated in 1948 by G. Harris (1948). Beginning in 1969, several hypothalamic peptides controlling pituitary function were isolated by A. Schally and R. Guillemin (Schally *et al.* 1973). Corticotropin releasing hormone (CRH) was one of the last to be isolated and sequenced in 1981 by W. Vale, J. Spiess, C. Rivier and J. Rivier (1981).

For CRH as well as for the other peptides, it soon became apparent, that they were even more abun-

dant in other central nervous system nuclei, outside the hypothalamic unit, and that they could modulate neuron function (Guillemin 1978). CRH, for instance, modulates the sleeping-waking cycle, sustained attention, memory, learning, aggressiveness, anxiety, locomotor, eating and sexual behaviour, maternal behaviour and lactation, and induces analgesia (Sutton *et al.* 1982; Krahn *et al.* 1988).

Direct measurement of central neuropeptides is not possible in human subjects, except by sampling the cerebrospinal fluid. Although many of the neuropeptides can also be found in the peripheral circulation, two kinds of argument have hindering the development in this area.

First, these peptides are also produced by several peripheral organs, namely the gut, and it would be impossible to differentiate between a central and peripheral origin (Muglia *et al.* 1994). Secondly it is generally assumed that the brain-blood barrier, prevents central neuropeptides to reach the peripheral circulation (Kastin *et al.* 1990).

None of these arguments stands a closer inquiry. Neuropeptides are much more abundant in the central nervous system in absolute terms (Muglia *et al.* 1994; Swanson *et al.* 1983). Specific, sophisticated and modulated transport systems exist in the blood-brain barrier that allow for the movement of peptides in either direction (Kastin *et al.*, 1990; Kastin *et al.* 1999).

In particular for CRH, studies in mice, have shown that there is a specific and modulated transport system for the rapid movement of CRH out of the central compartment (Martins *et al.* 1996; Martins *et al.* 1997a). Because of this transport system, the central CRH pool explains peripheral CRH levels almost completely (Martins *et al.* 1997b). Furthermore, central CRH from outside the hypothalamic-pituitary unit is able to reach and act at peripheral organs, while no effect is apparent if the specific brain to blood transport system is inhibited (Martins *et al.* 1997b).

Plasma levels of neuropeptides may then be a reflection of central levels. This may allow for its use in the study of psychiatric disorders, and as a research tool for exploring the interplay between behaviour and disease states in clinical medicine.

Showing the acute behavioural modulation of plasma levels of neuropeptides, would be strong evidence for the above. That is what we purposed to do, by showing the acute modulation of plasma levels of CRH during the feeling of induced emotions. Additionally we explored the relation between baseline levels of CRH and conventional indexes of pituitary-adrenal gland function and behavioural traits.

PATIENTS AND METHODS

Sixty consecutive adult female patients with simple nodular goitre were included in the protocol. Patients were assisted at the endocrine out patient department of a public central hospital. They were selected at the first visit, before beginning any pharmacological treatment. Simple nodular goitre was defined by the sonographic demonstration of more than one thyroid nodule less than 1 cm in diameter, with negative thyroid auto antibodies (thyroglobulin and thyroid peroxydase antibodies) and normal thyroid function (T3, T4 and TSH and free T4). Written informed consent was obtained in every case and the research protocol was approved by the ethical department of the medical school. It must be emphasized that since the general use of sensitive sonographic methods, simple nodular goitre as defined, is extremely common and may be considered as a "non-pathologic condition". Since the study was conducted at a central public hospital, by necessity such subjects are generally chosen as controls, to equalize for the setting in regard to experimental subjects. No patient had previous evidence of any psychiatric disorder and no patient was under treatment with psychotropic or steroid drugs. For the sake of homogeneity, only female subjects were considered.

Patients were received in the Dynamic Testing Room of the Endocrine Unit at 3.00 p.m. after lunch. Three groups were defined: Group A – for the induction of fear/sadness emotion; Group B – for the induction of joy/happiness emotion; Group C – no emotion induction. Each group comprised 20 subjects. Groups of 2–3 subjects were studied each session, randomly for groups A, B or C.

The research protocol was again explained and height, weight, blood pressure and heart rate were measured. An antecubital vein was canulated and maintained patent by flushing with sodium chloride solution (0.9%) with sodium heparin (25 U/mL). Thirty min later, a basal venous blood sample was obtained (sample 1–10 mL). A chosen fragment of 20 min duration of a dramatic (group A) or comic (group B) content movie was projected in a video equipment; no movie was projected in group C. Ten min after the movie – 30 min after the baseline sample 1 – a new venous blood sample was obtained in all subjects including those of group C (sample 2–10 mL). Subjects then filled the Minnesota Multiphasic Personality Inventory (MMPI-1) and in groups A and B, 60 min later – 90 min after the baseline sample – a new venous blood sample was obtained (sample 3–10 mL). The venous catheter was removed, blood pressure and heart rate were again measured, patients were thanked and dismissed. No financial retribution was allocated.

Two commercial movies used were: Schindler's List by Steven Spielberg, Universal Studios, 1993 (dramatic content), the destruction of Warsaw ghetto, 53–73 min and The Amazing Adventures of Mr. Bean by Paul Weiland and John Birkin, USA Films, 1989 (comic content), 0–20 min.

Correction and validation of the Minnesota Multiphasic Personality Inventory (MMPI-1) was done as indicated by the booklet instructions (Perse 1986). To

avoid the use of multiple comparisons, only conventional superordinate traits, computed by adding t-scores of the appropriate clinical scales, were used in the analysis; these were the neurotic triad – hypochondria (Hs) + depression (D) + hysteria (Hy) – the psychotic dyad – schizophrenia (Sc) + paranoia (Pa) – and behaviour problems – masculinity-femininity (Mf) + hypomania (Ma) + psychopathic deviate (Pd) (Greene 1991).

Venous blood samples were collected in lithium heparin tubes and maintained at +4 °C for the next 4 h. Six mL of blood were then transferred to new tubes with aprotinin (0.3 U) and centrifuged at 10,000 rpm for 5 min at +4 °C. Plasma was stored at –70 °C until the assay (within 30 days).

CRH was measured by enzyme-linked immunosorbent assay (EIA). Commercially available diagnostic kits were used (Pensinsula Laboratories, San Carlos, CA). All samples were assayed in duplicate. All measurements regarding the same patient, were made in the same laboratorial session. Random samples were also assayed in duplicate between 1–6 months after the first assay.

Routine and endocrine measurements, deemed appropriate in each case, were determined by automatic routine methods, and are not further described since they are not fundamental for this work. This includes ACTH, cortisol, and DHEAS (ELISA) (Diagnostic Products Corporation, Los Angeles, CA).

Results are expressed as the mean ± standard deviation or as % as appropriate. The normal distribution of continuous variables was verified by the Kolmogorov-Smirnov test. Non-normal distributed variables were log transformed prior to analysis; for the sake of simplicity however, when no differences were found, results regarding the non-transformed variables are presented. Statistical analysis used the Statistical Package for the Social Sciences Program (SPSS, Chicago, IL). Repeated-measures ANOVA were used to compare the results at different times, with post-hoc paired t-Student tests. Multiple regression analysis was used to explore the relation between continuous variables. 0.05 was used as the limit of statistical significance.

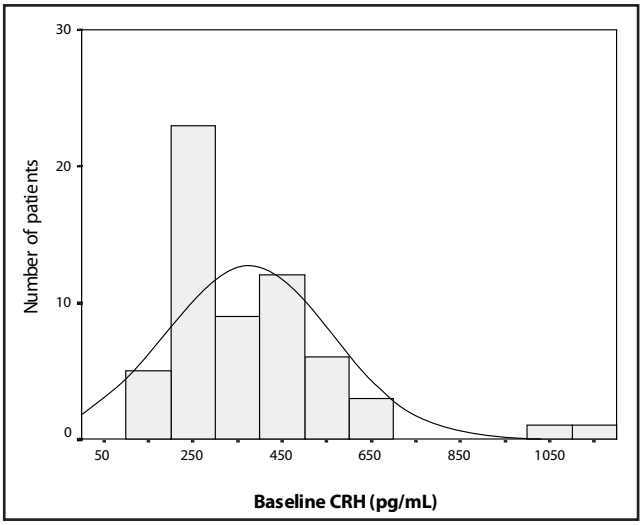

Fig. 1. Distribution of baseline CRH values. The best fitted, computer designed normal curve is also presented.

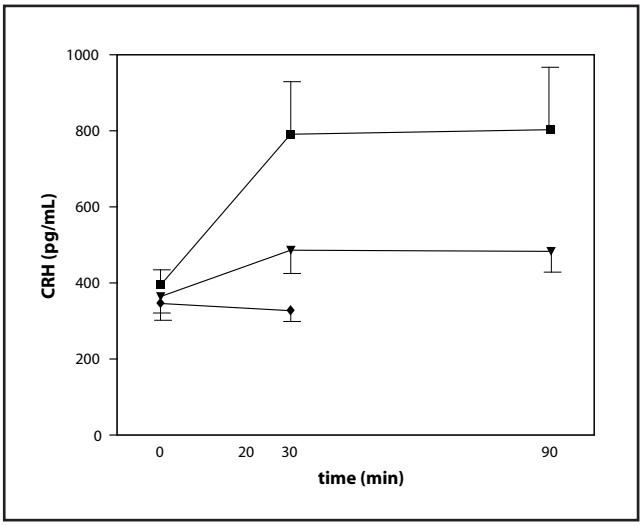

Fig. 2. CRH values in the three experimental groups. Group A - black squares; Group B - black triangles; group C - black diamonds. For each time moment, the mean+standard error of the mean or the mean-standard error of the mean is presented.

RESULTS

Baseline CRH results were 365±178 pg/mL [191–1100], median – 312 pg/mL, n=60. The variable presented a distribution that was not significantly different from the normal one, Kolmogorov-Smirnov Z – 1.292, $p<0.10$, skeweness – 1.927, kurtosis – 5.039 (Figure 1). Intra- and interassay coefficients of variation were 12% and 18% respectively. The sensitivity limit was 220 pg/mL. The linear range for CRH measurement was between 160–5,000 pg/mL. According to the manufacturer, cross-reactivity with other forms of CRH (ovine and bovine) and prepro CRH is less than 1%.

Control values, with no emotion induction, were obtained from group C (Figure 2). There were no significant differences between samples 1 and 2 in this group – 346±198 vs. 327±143 pg/mL respectively, paired samples t=0.52, df=19, $p=0.6$. Values decreased from sample 1 to sample 2 in 11 subjects and increased in 9 subjects. Differences were greater than 10% in 13 subjects, and greater than 20% in 8 subjects. Values from samples 1 and 2 were directly and significantly related – n=20, r=0.495, $p<0.05$.

Results regarding the induction of fear/sadness emotion were obtained from group A (Figure 2). Values for samples 1,2 and 3 were 394±147, 791±636 and 803±771 pg/mL respectively. Repeated-measures ANOVA showed a significant time effect – $F(2,57)=4.399$, $p<0.05$. Paired sample analysis showed significant differences between samples 1 and 2 – t=2.76, df=19, $p<0.05$ – and samples 1 and 3 – t=2.37,

df=19, $p<0.05$. Values increased from sample 1 to sample 2 in 14 subjects and decreased in 6. Increases were greater than 10% in 12 subjects, and greater than 20% in 10 subjects. Baseline levels were significantly related to peak levels (sample 2) – n=20, r=0.469, $p<0.05$ – and these were significantly related with each other (samples 2 and 3) – n=20, r=0.538, $p<0.05$.

The induction of joy/happiness emotion was ascertained in group B (Figure 2). Values for samples 1,2 and 3 were 364±138, 486±260 and 483±228 pg/mL. Repeated-measures ANOVA showed a trend for time effect – F(2,57)=2.680, $p<0.10$. Paired sample analysis showed a trend for differences between samples 1 and 2 – t=1.87, df=19, $p<0.10$ – and significant differences between samples 1 and 3 – t=2.53, df=19, $p<0.05$. From sample 1 to sample 2, values increased in 13 subjects, and decreased in 7; increases were greater than 10% in 13 subjects and greater than 20% in 10 subjects. Baseline levels were significantly related to peak levels (sample 2) – n=20, r=0.491, $p<0.05$ – and peak levels were significantly related to each other (samples 2 and 3) – n=20, r=0.565, $p<0.05$.

Baseline CRH levels were not significantly related to either ACTH, cortisol or DHEAS (data not shown).

Results from the psychometric evaluation were valid in all cases judging by validity scales unanswered (?), lie (L), fake (F) and correction bias (K). Multiple regression analysis, revealed that baseline CRH was significantly related both to the neurotic triad – inverse relation, partial r=0.328, n=60, $p<0.05$ – and to the psychotic dyad – direct relation, partial r=0.267, n=60, $p<0.05$ (Figure 3).

DISCUSSION

Central neuropeptides, including hypothalamic regulatory peptides, modulate neuronal function, besides controlling pituitary activity (Guillemin 1978). CRH, for instance deeply affects behaviour, and has been implicated in several neuropsychiatric disorders, like alcohol and drug addiction, dementia and depression (Wand & Dobs 1991; Milanes *et al.* 1998; Lupien *et al.* 1998; Gold *et al.* 1986). By simultaneously controlling pituitary function, vegetative functions and behaviour, CRH and other neuropeptides must intervene in the puzzling mind-body integration of classical authors.

Measurement of central neuropeptides, may therefore be instrumental to the understanding of psychiatric disorders, and useful to explore the behavioural component of common medical disorders, like obesity, high blood pressure, reproductive disturbances, immune-mediated disorders, and more generally in any acute and chronic stressful conditions (Rosmond *et al.* 1998; Chrousos *et al.* 1998; Sternberg *et al.* 1992; Chrousos & Gold 1992; Van den Bergh *et al.* 1998).

Direct measurement of central neuropeptides is not feasible at the clinical level. However, despite common belief to the contrary, plasma levels of central neuropeptides may be informative regarding central levels. This is so because the central pool is the major contributor to plasma levels and because specific, modulated transport systems exist across the brain-blood barrier (Martins *et al.* 1996; Martins *et al.* 1997a; Martins *et al.* 1997b; Sasaki *et al.* 1987; Wittert *et al.* 1993; Yanovski *et al.* 1998).

In this study we show the acute change in plasma levels of CRH, associated with specific mind states, following the experimental induction of specific and opposing emotions. At a different level, this study may be approached to other studies regarding the imaging of regional central nervous system activation or common endocrine responses during the feeling of induced emotions (Damasio *et al.* 2000; Sobrinho *et al.* 2003).

Taking into account the plasma half-life of CRH, no significant changes were found in plasma levels

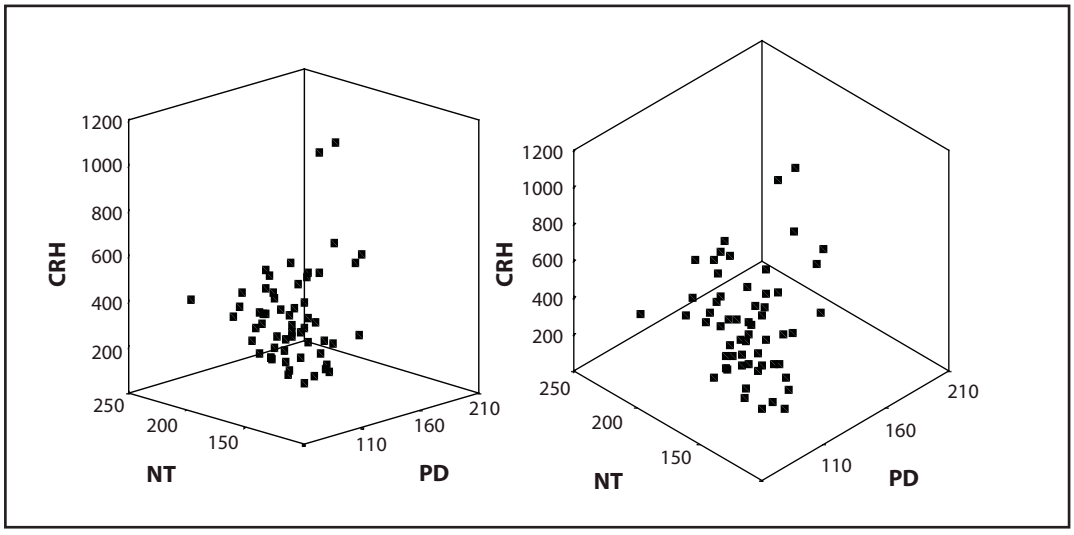

Fig. 3. Bivariate regression analysis of the neurotic triad score (NT) and the psychotic dyad score (PD) against baseline CRH (pg/mL). The 3D-graph is presented as viewed from the front (left) and from above (right).

between 30–60 min, after venous puncture, in resting subjects. However the experimental induction of fear/sadness emotion is associated with a clear and persistent increase in plasma CRH levels, persisting until the last sampling time 90 min after stimulus presentation. In other subjects, the induction of the opposing emotion of joy/happiness was associated with a less dramatic increase in plasma CRH levels, with a trend for significance.

These results suggest plasma CRH changes, result from fluctuations in central levels. We think no other explanation can account for the present findings. Physical stress, due for instance to venous puncture, cannot explain the different findings in the control, fear/sadness and joy/happiness groups. By the same token, filling the psychometric inventories cannot also account for the differential effects. It is theoretically possible, that autonomic nervous system function modulates the peripheral release of CRH, but it would be rather indirect to invoke gut and/or spleen release of CRH during the feeling of induced emotions. Furthermore such vegetative changes should be expected following venous puncture and no changes on plasma CRH levels were recorded in group C. Besides, previous evidence of specific transport systems across the brain-blood barrier and the much greater central pool of CRH clearly favours the first mechanism.

Baseline CRH levels were not significantly related to either ACTH, cortisol or DHEAS. This is not unexpectedly and suggests that the control of the pituitary-adrenal axis depends on CRH produced at the paraventricular hypothalamic nuclei that reaches the pituitary at very high levels through the portal hypothalamic-pituitary vessels. Previous studies were conducted regarding plasma CRH levels in Cushing's syndrome and disease and Addison's disease (Sasaki *et al.* 1987; Wittert *et al.* 1993; Yanovski *et al.* 1998). These studies did not find significant differences regarding plasma CRH levels in those conditions, when compared to control groups. This is not surprisingly since hypothalamic CRH levels should be a minor contributor to total central nervous system CRH levels and therefore to plasma CRH levels. More recently plasma CRH levels were not affected by dexamethasone treatment, again suggesting an extra-hypothalamic origin (Galard *et al.* 2002).

To the best of our knowledge no other published study explores plasma levels of CRH in relation to emotions. Pertinent to present findings, may be some previous studies regarding cerebrospinal fluid CRH levels in control subjects and subjects with major depression (Nemeroff *et al.* 1984; Geracioti *et al.* 1992). Increased CRH levels were found, supporting the hypothesis that behavioural conditions affect central CRH levels, outside the hypothalamic unit, and in agreement with present findings of a significant direct relation between plasma CRH and the psychotic dyad score. Also, increased plasma CRH levels were found in patients with major depression and posttraumatic stress disorder (Catalán *et al.* 1998; Galard *et al.* 2002; Kloet *et al.* 2008).

The postulated central origin of plasma CRH, besides the acute changes following emotion induction is also suggested by the significant relation with some behavioural traits. Baseline CRH was clearly related both to the neurotic triad and to the psychotic dyad superordinate traits, in this sample of non-psychiatric subjects. Interestingly enough the relation with the neurotic triad was inverse, while that with the psychotic dyad was direct. Up to the point the relation between hypothalamic-pituitary-adrenal axis reactivity – assessed by CRH testing – and neurotic traits or psychotic conditions has already been reported (Gold *et al.* 1986a; Gold *et al.* 1986b; Martins *et al.* 2001; Martins *et al.* 2002). As noted the same was shown for plasma CRH (Catalán *et al.* 1998; Galard *et al.* 2002; Kloet *et al.* 2008).

Plasma levels of CRH now reported are higher than in other studies (Catalán *et al.* 1998). The difference may be accounted for, by three general factors: we measured total and not free CRH; we did not use previous extraction; and we used an enzyme-linked immunosorbent assay instead of an immunoradiometric assay. The possibility of some cross-reaction with urocortin or other CRH-like peptides can not be completely discarded, even if this is highly unlikely, considering the peripheral circulation, and the specific setting, i.e., after emotion induction.

In short we showed in this paper acute and persistent plasma changes in CRH levels associated with the induction of specific emotions. Additionally, we showed baseline CRH to be significantly related to some behavioural traits. Taken together and with other evidence, this strongly suggests that plasma levels of CRH, and possibly of other central neuropeptides, reflect central levels, and may be used to explore the complex relations between behaviour and physical illness.

REFERENCES

1 Catalán R, Gallart JM, Castellanos JM, Galard R. (1998). Plasma corticotropin-releasing factor in depressive disorders. Biol Psychiatry. **44**: 15–20.
2 Chrousos GP, Gold PW. (1992). The concepts of stress and stress system disorders. JAMA. **267**: 1244–1252.
3 Chrousos GP, Torpy DJ, Gold PW. (1998). Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. Ann Intern Med. 129: 229–240.
4 Damásio AR, Grabowski TJ, Bechara A, Damásio H, Ponto LLB, Parvizi J, *et al.* (2000). Subcortical and cortical brain activity during the feeling of self-generated emotions. Nature Neurosci. **3**: 1044–1056.
5 Galard R, Catalán R, Castellanos JM, Gallart JM. (2002). Plasma corticotropin-releasing factor in depressed patients before and after the dexamethasone suppression test. Biol Psychiatry. **15**: 463–8.
6 Geracioti TD, Orth DN, Ekhator NN, Blumenkopf B, Loosen PT. (1992). Serial cerebrospinal fluid corticotropin-releasing hormone concentrations in healthy and depressed humans. J Clin Endocrinol Metab. **74**: 1325–1330.

7 Gold PW, Loriaux L, Roy A, Kling MA, Calabrese JR, Kellner CH, *et al.* (1986). Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease. N Engl J Med. **314**: 1329–1335.
8 Gold PW, Gwirtsman H, Avgerinos PC, Nieman LK, Gallucci WT, Kaye W, *et al.* (1986). Abnormal hypothalamic-pituitary-adrenal function in anorexia nervosa. N Engl J Med. **314**: 1335–1342.
9 Greene RL. (1991). The MMPI-2/MMPI: an interpretive manual. Boston: Allyn & Bacon.
10 Guillemin R. (1978). Peptides in the brain: the new endocrinology of the neuron. Science. **202**: 390–402.
11 Harris GW. (1948). Neural control of the pituitary gland. Physiol Rev. **28**: 139–179.
12 Kastin AJ, Banks WA, Zadina JE. (1990). A decade of changing perceptions about neuropeptides. Ann NY Acad Sci. **579**: 1–7.
13 Kastin AJ, Pan W, Maness LM, Banks WA (1999). Peptides crossing the blood-brain barrier: some unusual observations. Brain Res. **848**: 96–100.
14 Kloet CS, Vermetten E, Geuze E, Lentjes EG, Heijnen CJ, Stalla GK, *et al.* (2008). Elevated plasma corticotrophin-releasing hormone level in veterans with posttraumatic stress disorder. Prog Brain Res. 167: 287–291.
15 Krahn DD, Gosnell BA, Levine AS, Morley JE. (1988). Behavioral effects of corticotropin-releasing factor: localization and characterization of central effects. Brain Res. **443**: 63–69.
16 Lupien S, Tchiteya BM, Maheu F, Sharma S, Nair NPV, Hauger RL, *et al.* (1998). Stress-induced declarative memory impairment in healthy elderly subjects: relationship to cortisol reactivity. J Clin Endocrinol Metab. **82**: 2070–2075.
17 Martins JM, Kastin AJ, Banks WA. (1996). Unidirectional specific and modulated brain to blood transport of corticotropin-releasing hormone. Neuroendocrinology. **63**: 3338–3348.
18 Martins JM, Banks WA, Kastin AJ. (1997). Acute modulation of active carrier-mediated brain-to-blood transport of corticotropin-releasing hormone. Am J Physiol. **272**: E312–E319.
19 Martins JM, Banks WA, Kastin AJ. (1997). Transport of CRH from mouse brain directly affects peripheral production of β-endorphin by the spleen. Am J Physiol. **273**: E1083–E1089.
20 Martins JM, Trinca A, Afonso A, Carreiras F, Falcão J, Nunes JS, *et al.* (2001). Psychoneuroendocrine characteristics of common obesity clinical subtypes. Int J Obesity. **25**: 24–32.
21 Martin Martins J, Alves J, Trinca A, Grima B, do Vale S, Vasconcelos T, *et al.* (2002). Personality, brain asymmetry, and neuroendocrine reactivity in two immune-mediated disorders: a preliminary report, Brain Behav Immunity. **16**: 383–397.
22 Milanes MV, Laorden ML, Chapleur-Chateau M, Burlet A. (1998). Alterations in corticotropin-releasing factor and vasopressin content in rat brain during morphine withdrawal: correlation with hypothalamic noradrenergic activity and pituitary-adrenal response. J Pharmacol Exp Ther. **285**: 700–706.
23 Muglia LJ, Jenkins NA, Gilbert DJ, Copeland NG, Majzoub JA. (1994). Expression of the mouse corticotropin-releasing hormone gene in vivo and targeted inactivation in embryonic stem cells. J Clin Invest. **93**: 2066–2072.
24 Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, *et al.* (1984). Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. Science. **226**: 1342–1344.
25 Perse J. (1986). Manuel Inventaire Multiphasique de Personnalité du Minnesota (M.M.P.I.). Paris : Les Editions du Centre de Psychologie Appliquée.
26 Rosmond R, Dallman MF, Bjorntorp P. (1988). Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. J Clin Endocrinol Metab. **83**: 1853–1859.
27 Sasaki A, Sato S, Murakami O, Go M, Inoue M, Shimizu Y, *et al.* (1987). Immunoreactive corticotropin-releasing hormone present in human plasma may be derived from both hypothalamic and extrahypothalamic sources. J Clin Endocrinol Metab. **65**: 176–182.
28 Schally AV, Arimura A, Kastin AJ. (1973). Hypothalamic regulatory hormones. Science. **179**: 341–350.
29 Sobrinho LG, Simões M, Barbosa L, Raposo JF, Pratas S, Fernandes PL, *et al.* (2003). Cortisol, prolactin, growth hormone and neurovegetative responses to emotions elicited during an hypnoidal state. Psychoneuroendocrinol. **28**: 1–17.
30 Sternberg EM, Young WS, Bernardini R, Calogero AE, Chrousos GP, Gold PW, *et al.* (1989). A central nervous system defect in biosynthesis of corticotropin-releasing hormone is associated with susceptibility to streptococcal cell wall-induced arthritis in Lewis rats. Proc Natl Acad Sci USA. **86**: 4771–4775.
31 Sutton RE, Koob GF, Rivier J, Vale W (1982). Corticotropin releasing factor produces behavioural activation in rats. Nature. **297**: 331–333.
32 Swanson LW, Sawchenko PE, Rivier J, Vale W. (1983). Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology. **36**: 165–186.
33 Vale W, Spiess J, Rivier C, Rivier J. (1981). Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. Science. **213**: 1394–1397.
34 Van den Berghe G, De Zegher F, Bouillon R. (1998). Acute and prolonged critical illness as different endocrine paradigms. J Clin Endocrinol Metab. **83**: 1827–1834.
35 Wand GS, Dobs AS. (1991). Alterations in the hypothalamic-pituitary-adrenal axis in actively drinking alcoholics. J Clin Endocrinol Metab. **72**: 1290–1295.
36 Wittert GA, Livesey JH, Florkowski C, Or HK, Donald RA, Espiner EA. (1993). Acutely raised corticotropin levels in Addison's disease are not associated with increased plasma arginine vasopressin and corticotropin-releasing factor concentrations in peripheral plasma. J Clin Endocrinol Metab. **76**: 192–196.
37 Yanovski JA, Nieman LK, Doppman JL, Chrousos GP, Wilder RL, Gold PW, *et al.* (1998). Plasma levels of corticotropin-releasing hormone in the inferior petrosal sinuses of healthy volunteers, patients with Cushing's syndrome, and patients with pseudo-Cushing states. J Clin Endocrinol Metab. **83**: 1485–1488.