

Regulation of corticosteroid receptors in patients with anorexia nervosa and Cushing's syndrome

D Armanini, P Spinella¹, M Simoncini, A Basso³, S Zovato, G B Pozzan², C B De Palo, G Bucciante² and I Karbowski⁴

Istituto Semeiotica Medica, ¹Cattedra Nutrizione Clinica and ²Dipartimento di Pediatria, University of Padua, ³Divisione Malattie del Ricambio, Ospedale S Bortolo Vicenza and ⁴Laboratorio Analisi, Ospedale Piove di Sacco, Padua, Italy

(Requests for offprints should be addressed to D Armanini, Istituto di Semeiotica Medica, University of Padua, Via Ospedale 105, 35100 Padua, Italy)

Abstract

We have studied 16 patients with anorexia nervosa (11 with a stabilised weight loss and 5 in the weight-losing phase), 11 healthy controls, and 10 patients with Cushing's syndrome, by measuring plasma cortisol (by enzyme-immunoassay), ACTH (by RIA), corticosteroid (Type I-mineralocorticoid and Type II-glucocorticoid) receptors in mononuclear leukocytes (by radio-receptor assay), and lymphocyte subpopulations (by cytofluorimetry).

In anorexic patients with a stabilised weight loss and in Cushing's syndrome the mean value of both Type I and Type II corticosteroid receptors in mononuclear leukocytes was significantly lower than in controls. The corre-

lation between Type II receptors and plasma cortisol was inverse in stabilised anorexia nervosa and in Cushing's syndrome, and direct in healthy controls. Anorexic patients in the weight-losing phase showed a significant increase in plasma cortisol levels and a normal number of Type II receptors.

From these results we hypothesise that in anorexia nervosa there is a progression from an increase in plasma cortisol in the weight-losing phase, to a concomitant decrease in Type II receptors when the disease is stabilised.

Journal of Endocrinology (1998) **158**, 435–439

Introduction

Anorexia nervosa and Cushing's syndrome are associated with increased levels of plasma cortisol (Walsh *et al.* 1987, Vierhapper *et al.* 1990). A common pattern is also the frequent lack of response to an overnight dexamethasone suppression test (Walsh *et al.* 1987, Vierhapper *et al.* 1990, Seeman & Robbins 1994). The effects of cortisol are mediated by binding to corticosteroid receptors at the level of target tissues. The corticosteroid receptors are of two types, Type I and Type II (Funder *et al.* 1988, Funder 1996). Type I receptors are bound by aldosterone in tissues in which cortisol is inactivated to cortisone by the Type II 11-hydroxysteroid dehydrogenase (Funder 1996), while in other tissues which lack the enzyme, as for example in the rat hippocampus, Type I receptors act as glucocorticoid receptors, being bound by cortisol, and are also involved in the regulation of corticotrophin-releasing factor–adrenocorticotrophin (ACTH)–cortisol feedback (Bradbury *et al.* 1991). Type I receptors are also targets for mineralocorticoids in non-classical target tissues for aldosterone when cortisol and/or aldosterone are increased (Wehling *et al.* 1987). Type II receptors do respond to higher concentrations of cortisol such as those early in the morning or during stress (Bradbury *et al.* 1991). A continuous occupancy of corticosteroid receptors results in

excessive activity of steroid-responsive genes despite the capacity for a down-regulation of the receptors which can blunt the effector mechanism itself (Bradbury *et al.* 1991). The brain corticosteroid receptors are inaccessible in the clinical population, therefore the study of corticosteroid receptors in humans must be indirect. In the adrenalectomised rat the regulation of corticosteroid receptors is similar in lymphoid tissue and hippocampus (Lowy 1991), supporting the utilisation of mononuclear leukocytes (MNL) as an index of the regulation of corticosteroid receptors at the supra-hypothalamic level in humans. In a previous study we have characterised Type I receptors in MNL (Armanini *et al.* 1985a) and have found an equal amount of Type I and Type II receptors in T- and B-lymphocytes (Armanini *et al.* 1988). The increased cortisol in anorexia nervosa is not associated with clinical symptoms of hypercortisolism and the number of Type II receptors was found to be normal (Girardin *et al.* 1991) or reduced (Kontula *et al.* 1982) in these patients. We have evaluated the corticosteroid Type I and Type II receptors in MNL and lymphocyte subpopulations in patients with anorexia nervosa and Cushing's syndrome, a clinical situation where the effector mechanism of cortisol is clearly increased. The evaluation of lymphocyte subpopulations could provide some information on the lymphoid system changes in hypercortisolism. It has been shown that oral

Table 1 Characteristics of the patients with anorexia nervosa group 1, anorexia nervosa group 2, Cushing's syndrome and the control group. Values are means \pm s.d.

	Anorexia nervosa (1)	Anorexia nervosa (2)	Cushing	Control
Age	20 \pm 4	16 \pm 3	36 \pm 10	24 \pm 9
BMI	15.2 \pm 1.2	15 \pm 1	31 \pm 2	22 \pm 1
Plasma cortisol (μ g/dl)*	24.5 \pm 6.5	22 \pm 0.7	30.3 \pm 5.1	12.9 \pm 3.5
Type I receptor ($n \times$ cell)	125 \pm 78	124 \pm 70	120 \pm 18	284 \pm 85
Affinity (nM)	1.9 \pm 0.2	2 \pm 0.3	2.1 \pm 0.3	1.9 \pm 0.2
Type II receptor ($n \times$ cell)	1523 \pm 654	3920 \pm 1052	2435 \pm 768	3813 \pm 892
Affinity (nM)	7.9 \pm 2.0	7.8 \pm 2.9	7.6 \pm 2.4	7.9 \pm 0.3
CD4 (%)	41 \pm 8	40 \pm 1.2	40 \pm 9	48 \pm 8
CD8 (%)	26 \pm 5	30 \pm 4	31 \pm 4	30 \pm 4
CD4/CD8	1.6 \pm 0.5	1.3 \pm 0.6	1.3 \pm 0.5	1.6 \pm 0.5

*To convert to micromoles per litre, multiply by 0.02759.

administration of prednisone decreases the number of CD4 (T helper lymphocytes) lymphocytes in blood (Westermann & Pabst 1990).

Materials and Methods

Patients

Sixteen cases of anorexia nervosa, fourteen females and two males (age 12–27 years), and ten subjects with Cushing's syndrome (two unilateral adrenal adenomas and eight patients with Cushing's disease, mean age 36 \pm 10 years), were studied. The anorexic patients were all pure food restrictors and were without signs of other complicating diseases. They were subdivided into two groups in relation to the phase of the disease: 11 out-patients, anorexic for more than two years with a stable maintenance of the \geq 25% level of the previous weight (group 1; 10 females, 1 male, age 20 \pm 4 years, body mass index (BMI) 15.2 \pm 1.2) and 5 in-patient subjects with emaciation and progressive weight loss (group 2; 4 females, one male, age 16 \pm 3 years, BMI 15 \pm 1). All the anorexic patients met the criteria of the Diagnostic and Statistical Manual IV-R for anorexia nervosa (weight loss of at least 25% of the pre-disease weight, no other diseases which could produce weight loss, refusal to maintain adequate body weight, psychological disturbances) (American Psychiatric Association 1993). The three youngest subjects had primary and the other women had secondary amenorrhea for at least six months. All the subjects studied were free of therapies and in particular none were taking oestrogens nor progestins. A control group of 11 subjects, sex- and age-matched with the anorexic group 1, with normal weight and no history of anorexia nervosa was also studied. Table 1 shows the main characteristics of the patients. In all cases a blood sample was drawn at 0900 h for measurement of plasma cortisol, Type I and Type II receptors numbers in MNL, and lymphocyte subpopu-

lations. Plasma ACTH was measured in 6 cases with group 1 anorexia nervosa, in all cases with group 2 anorexia nervosa and in all cases with Cushing's syndrome. All subjects were clearly informed of the study and gave their consent.

Methods

Plasma cortisol was measured by enzyme-immunoassay (TDX; Abbott, Rome, Italy), plasma ACTH was measured by RIA (Euro Diagnostic, Harnhem, Holland; range of normality 20–100 pg/ml (4.4–22.2 pmol/l)), corticosteroid receptors were measured in MNL by a radio-receptor assay as previously described (Armanini *et al.* 1985a,b). Briefly the MNL were separated from the heparinised blood by a Percoll gradient and the cells were preincubated three times in saline and centrifuged in order to dissociate endogenous steroids from the receptors. An aliquot of cells was then incubated for one hour at 37 °C, with increasing amounts of [³H]aldosterone or [³H]dexamethasone alone or with addition of excess of the respective cold hormones. After incubation and washings the radioactivity was counted and results expressed by Scatchard analysis. In the Type I assay an excess of a pure glucocorticoid RU 26988 was added to block aldosterone binding to Type II receptors. The normal range in adult subjects for Type I receptors is 175–400 and for Type II receptors 2400–8000 receptors per cell. Lymphocyte subpopulations were measured by cytofluorimetry (Cytoron Ortho, Milan, Italy), using specific antibodies. Results are expressed as single values and means \pm s.d. Statistical analysis was done by Scheffe *f*-test preceded by an ANOVA.

Results

The mean value of plasma cortisol was significantly higher in anorexic patients (group 1) and in Cushing's syndrome than in controls (anorexia 24.5 \pm 6.5, Cushing 30.3 \pm 5.1,

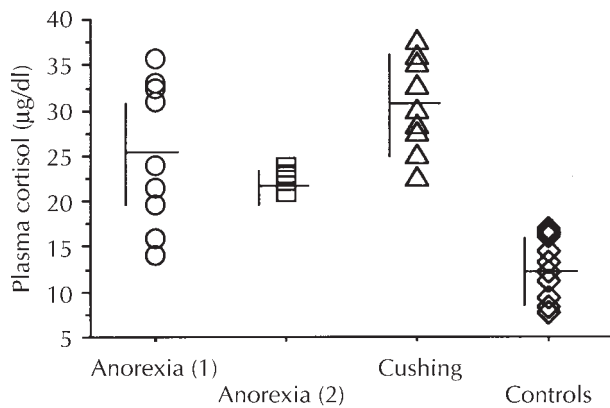


Figure 1 Plasma cortisol concentration (single values and means \pm s.d.) in patients with anorexia nervosa group 1 (with stabilised weight loss, $n=11$), anorexia nervosa group 2 (in the phase of emaciation, $n=5$), Cushing's syndrome ($n=10$) and in controls ($n=11$).

controls 12.9 ± 3.5 $\mu\text{g/dl}$, $P < 0.0001$, Fig. 1). The numbers of Type I and Type II receptors per cell were significantly lower in group 1 anorexia nervosa (125 ± 78 and 1523 ± 654 receptors per cell respectively) than in controls (284 ± 85 , $P < 0.0003$ and 3813 ± 892 , $P < 0.0001$ respectively, Figs 2 and 3). In Cushing's syndrome the number of Type I and Type II receptors (120 ± 18 and 2435 ± 768 respectively) was lower than in controls ($P < 0.05$ and $P < 0.02$ respectively), but Type II receptor numbers were above the range of normality only in three cases (Figs 2 and 3). Plasma ACTH values were in the normal range in both group 1 (mean 76 ± 13 pg/ml, 16.7 ± 2.8 pmol/l, $n=7$) and group 2 (mean 70 ± 19 pg/ml, 15.4 ± 4.2 pmol/l, $n=5$) anorexia nervosa. No difference was observed in the affinity of [^3H]aldosterone and [^3H]dexamethasone for the respective receptors in all the groups studied (Table 1). The percentage of CD4 (T

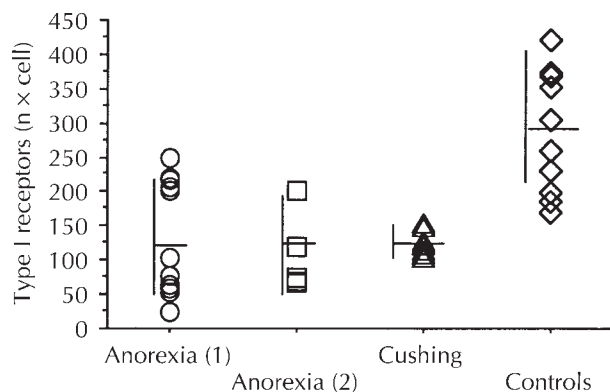


Figure 2 Numbers of Type I corticosteroid receptors (single values and means \pm s.d.) in mononuclear leukocytes of patients with anorexia nervosa group 1 (with stabilised weight loss, $n=11$), anorexia nervosa group 2 (in the phase of emaciation, $n=5$), Cushing's syndrome ($n=10$) and in controls ($n=11$).

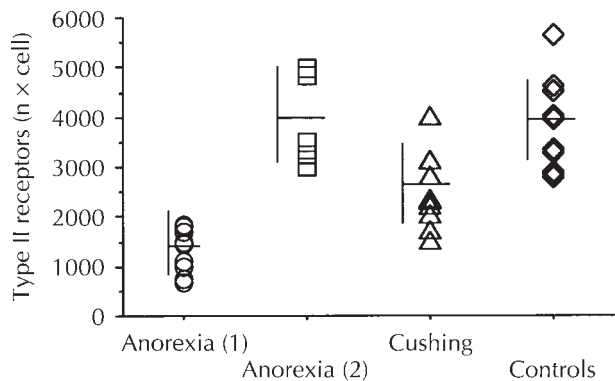


Figure 3 Numbers of Type II corticosteroid receptors (single values and means \pm s.d.) in mononuclear leukocytes of patients with anorexia nervosa group 1 (with stabilised weight loss, $n=11$), anorexia nervosa group 2 (in the phase of emaciation, $n=5$), Cushing's syndrome ($n=10$) and in controls ($n=11$).

helper lymphocytes) and CD8 (T suppressor lymphocytes) subsets and the CD4/CD8 ratios were in the normal range, and not different in the three groups (anorexia 1.6 ± 0.5 , Cushing 1.3 ± 0.5 and controls 1.6 ± 0.5) (Table 1). A positive significant correlation was found between plasma cortisol and Type II receptors in controls ($P < 0.03$), while this correlation was inverse in group 1 anorexia nervosa ($P < 0.0005$, Fig. 4) and in Cushing's syndrome ($P < 0.0005$, Fig. 4). No significant correlations were found between plasma cortisol and Type I receptors, nor between Type I and Type II receptors. In the group 2 anorexic patients plasma cortisol was higher (mean 22 ± 0.7 $\mu\text{g/dl}$, $P < 0.03$) and Type I receptor numbers were lower (124 ± 70 receptors per cell, $P < 0.001$) when compared with controls, while Type II receptor numbers (3920 ± 1052 receptors per cell) (Figs 1, 2 and 3) and lymphocyte subpopulations were in the normal range (Table 1).

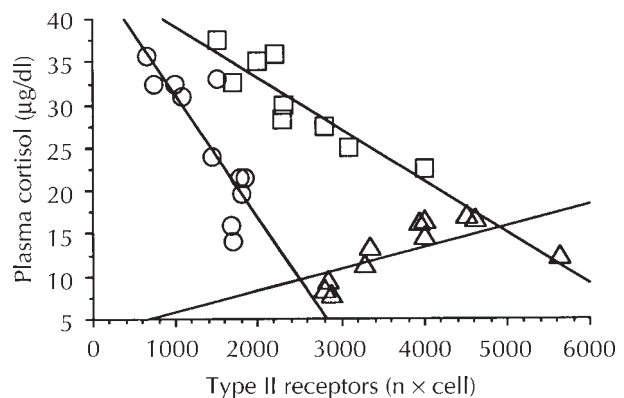


Figure 4 Significant correlation between plasma cortisol and Type II corticosteroid receptors in mononuclear leukocytes of patients with anorexia nervosa group 1 (\circ) ($r = -0.91$, $P < 0.0005$), Cushing's syndrome (\square) ($r = -0.89$, $P < 0.0005$) and in controls (\triangle) ($r = 0.60$, $P < 0.03$).

Discussion

Patients with malnutrition or psychiatric diseases frequently have high levels of plasma cortisol, and patients with Cushing's syndrome can also manifest psychiatric complications as a result. The exposure of cells in culture to glucocorticoids, or short-term administration of synthetic glucocorticoids, decreases the level of Type II corticosteroid receptors in MNL (Schlechte *et al.* 1982, Hampl *et al.* 1994), but the previous studies in patients with long-term corticosteroid treatment and Cushing's syndrome were not consistent with these findings, having corticosteroid receptor numbers still in the normal range in MNL (Pardes *et al.* 1989, 1991). The main results of our study are a more evident reduction of Type II corticosteroid receptors in MNL of patients with anorexia nervosa with stable weight loss than in patients with Cushing's syndrome, and an inverse correlation between plasma cortisol and Type II receptors in both groups. We have also confirmed our previous data of a positive correlation between plasma cortisol and corticosteroid Type II receptors in healthy subjects (Armanini *et al.* 1992). Our results in stabilised anorexia nervosa patients are in agreement with those of Kontula (1982) who used the same incubation temperature as in our study. It is worth noting that in the 5 cases who were still in the phase of body weight loss, the receptor number was normal, thus suggesting that the reduction of receptors is linked to the duration and the stabilisation of the disease and that the clinical manifestations precede the corticosteroid receptor abnormalities. Thus the mechanism for the reduction of corticosteroid receptors in group 1 anorexia nervosa seems more similar to that of aged subjects who have an age-related decrease of receptors and an increase of plasma cortisol to maintain an adequate function of the corticosteroid effector mechanism (Armanini *et al.* 1992, Armanini 1994). In fact we found both in ageing (Armanini 1994) and in group 1 anorexia nervosa an inverse correlation between plasma cortisol and Type II receptor number. We previously found a correlation between corticosteroid receptors and age (Armanini *et al.* 1992), but our anorexic subjects were an homogeneous group in relation to age and the data of group 1 anorexia nervosa was clearly lower than in controls. An inverse correlation between plasma cortisol and Type II receptors was also found in our patients with Cushing's syndrome, but in this case the receptor decrease was lower in relation to the respective value of plasma cortisol and this finding could be consistent with a subtle down-regulation of Type II receptors, as suggested also by Pardes *et al.* (1989). It has been demonstrated that immunodepression in Cushing's syndrome is mild suggesting an adaptation to hypercortisolism (Kling *et al.* 1993), and our data on lymphocyte subpopulations is consistent with this finding.

From our data it is not possible to demonstrate whether the reduction of corticosteroid receptors in our patients is

related to an insensitivity to glucocorticoid receptors (GR) or to a hyperfunction of the pituitary–adrenal axis. An hypothesis which could be formulated is that in initial anorexia nervosa there is an increase in plasma cortisol and that, after adaptation to the disease, there is also a reduction in the number of corticosteroid receptors at the hypothalamic, supra-hypothalamic and at the lymphoid level. Plasma ACTH concentration was not increased in our subjects, and our data is consistent with that of Gold *et al.* (1986), who hypothesised that the defect in anorexia is at, or above, the hypothalamus and not at the pituitary level. The clinical picture of frank hypercortisolism is not evident probably due to the prevalent reduction of corticosteroid receptors in relation to the respective cortisol values and to the lack of adequate metabolic substrate for the changes on fat disposition (Kling *et al.* 1993, Liu *et al.* 1994, Seeman & Robbins 1994).

A dysregulation in catecholaminergic regulation of corticosteroid receptors could be involved in the discrepancies between corticosteroid receptor number in the two groups of anorexic subjects. We did not measure catecholamines in our subjects but a previous study by Sato *et al.* (1988) has shown that subjects with anorexia nervosa with initial emaciation have adrenergic hyperactivity, while in the stabilisation phase the adrenergic tone is reduced. A possible alternative mechanism for modulation of glucocorticoid action could be an alternative splicing of the glucocorticoid receptor: GR β could act as a dominant negative inhibitor of GR α action (Ray 1996). Finally, the absence of clinical hypercortisolism in anorexia nervosa could be related to a decreased cortisol bioactivity under the control of 11 β -hydroxysteroid dehydrogenase activity modulating both glucocorticoid and mineralocorticoid hormone action (Funder 1996).

Another point of discussion is the implication of the reduction of Type I receptors in anorexia nervosa. A reduction of Type I receptors in MNL, with normal numbers of Type II receptors can be related to a down-regulation of the Type I receptors due to excess of mineralocorticoids as occurs in Conn's syndrome (Armanini *et al.* 1987). In anorexia nervosa the contemporary reduction of Type I and Type II receptors seems instead to be a primary phenomenon possibly linked to the same central mechanisms which reduce Type II receptors. In some of our anorexic patients with initial emaciation and in the patient who had a relapse of the disease, the Type I receptor number was reduced while the Type II receptor number was normal. This discrepancy is consistent with a prior reduction of Type I over Type II receptors.

In conclusion, from our data the course of anorexia nervosa is progressive and the reduction in corticosteroid receptors follows the increase in plasma cortisol; when corticosteroid Type II receptors decrease they are inversely correlated to plasma cortisol. The exact mechanism remains to be elucidated.

References

- American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, edn 4, Draft (DSM 4) 1993. Washington DC: APA Press.
- Armanini D 1994 Corticosteroid receptors in lymphocytes: a possible marker of brain involution? *Journal of Steroid Biochemistry and Molecular Biology* **49** 429–434.
- Armanini D, Strasser T & Weber PC 1985a Characterisation of aldosterone binding sites in human mononuclear leukocytes. *American Journal of Physiology* **248** E388–E390.
- Armanini D, Strasser T & Weber PC 1985b Parallel determination of glucocorticoid receptors in mononuclear and polymorphonuclear leukocytes after Percoll separation. *Journal of Endocrinological Investigation* **8** 45–47.
- Armanini D, Witzgall H, Wehling M, Kuhnle U & Weber P 1987 Aldosterone receptors in different types of primary aldosteronism. *Journal of Clinical Endocrinology and Metabolism* **65** 101–104.
- Armanini D, Enders S, Kuhnle U & Weber PC 1988 Parallel determination of mineralocorticoid and glucocorticoid receptors in T and B lymphocytes of human spleen. *Acta Endocrinologica* **118** 479–482.
- Armanini D, Karbowski I, Scali M, Orlandini E, Zampollo V & Vittadello G 1992 Corticosteroid receptors and lymphocyte subsets in mononuclear leukocytes in aging. *American Journal of Physiology* **282** E464–E466.
- Bradbury MJ, Akana SF, Cascio CS, Levin N, Jacobson L & Dallman MF 1991 Regulation of the basal ACTH secretion by corticosterone is mediated by both Type I (MR) and Type II (GR) receptors in rat brain. *Journal of Steroid Biochemistry and Molecular Biology* **40** 133–142.
- Funder JW 1996 Mineralocorticoid receptors and glucocorticoid receptors. *Clinical Endocrinology* **45** 651–656.
- Funder JW, Pearce P & Smith R 1988 Mineralocorticoid action target tissue specificity is enzyme not receptor mediated. *Science* **242** 583–587.
- Girardin E, Garosci-Cholet M, Dechaud H, Lejeune H, Carrier E, Tournaire J & Pugeat M 1991 Glucocorticoid receptors in lymphocytes in anorexia nervosa. *Clinical Endocrinology* **35** 79–84.
- Gold PW, Gwirtsman H, Avgerinos PC, Nieman NK, Gallucci BS, Kaye W, Jimerson D, Ebert M, Rittmaster R, Loriaux L & Chrousos P 1986 Abnormal hypothalamic–pituitary–adrenal function in anorexia nervosa. *New England Journal of Medicine* **314** 1335–1342.
- Hampl R, Lapcik O, Sulkova J, Starka L, Kalvachova B, Dvorakova M, Nemcova D & Hoza J 1994 The effect of long term glucocorticoid therapy on glucocorticoid receptor content and on steroid response to ACTH. *Journal of Steroid Biochemistry and Molecular Biology* **48** 81–87.
- Kling MA, Demitrack MA, Whitfield HJ Jr, Kalogeras KT, Listwak SJ, Debellis MD, Chrousos GP & Brandt HA 1993 Effect of the glucocorticoid antagonist RU486 on pituitary–adrenal function in patients with anorexia nervosa and healthy volunteers: enhancement of plasma ACTH and cortisol secretion in underweight patients. *Neuroendocrinology* **57** 1082–1091.
- Kontula A, Andersson LC, Huttunen M & Pelkonen R 1982 Reduced level of glucocorticoid receptors in patients with anorexia nervosa. *Hormone and Metabolic Research* **14** 619–620.
- Liu JP, Clarke IJ, Funder JW & Engler D 1994 Studies on the secretion of corticotrophin releasing factor and arginine vasopressin into the hypophysial portal circulation of the conscious sheep. The central noradrenergic or neuropeptide Y pathways cause immediate and prolonged hypothalamic–pituitary–adrenal activation. Potential involvement in the pseudo Cushing's syndrome of endogenous depression and anorexia nervosa. *Journal of Clinical Investigation* **93** 1439–1450.
- Lowy M 1991 Corticosterone regulation of brain and lymphoid corticosteroid receptors. *Journal of Steroid Biochemistry and Molecular Biology* **39** 147–154.
- Pardes EM, De Yampey JEW, Soto RJ, Moses DF & De Nicola AF 1989 Correlative study between glucocorticoid receptor levels in human mononuclear leukocytes and biochemical data in Cushing's disease. *Acta Endocrinologica* **120** 55–61.
- Pardes E, De Yampey JEW, Moses DF & De Nicola AF 1991 Regulation of corticosteroid receptors in human mononuclear cells: effects of glucocorticoid treatment, Cushing's syndrome and ketokonazole. *Journal of Steroid Biochemistry and Molecular Biology* **39** 233–238.
- Ray DW 1996 Molecular mechanisms of glucocorticoid resistance. *Journal of Endocrinology* **149** 1–5.
- Sato T, Igarashi N, Miyagava K, Nakajima T & Katayama K 1988 Catecholamine and thyroid metabolism in a case of anorexia nervosa. *Endocrinologia Japonica* **35** 295–301.
- Schlechte JA, Ginsberg BH & Sherman BM 1982 Regulation of the glucocorticoid receptor in human lymphocytes. *Journal of Steroid Biochemistry* **16** 69–74.
- Seeman TE & Robbins RJ 1994 Aging and hypothalamic–pituitary–adrenal response to challenge. *Endocrine Review* **15** 233–260.
- Vierhapper H, Kiss A, Nowotny P, Wiesnagrotzki S, Monder C & Waldhausl W 1990 Metabolism of cortisol in anorexia nervosa. *Acta Endocrinologica* **122** 753–758.
- Walsh BT, Roose SP & Katz JL 1987 Hypothalamic, pituitary and adrenal cortical activity in anorexia nervosa and bulimia. *Psychoneuroendocrinology* **12** 131–140.
- Wehling M, Armanini D, Strasser T & Weber PC 1987 Effect of aldosterone on sodium and potassium concentrations in human mononuclear leukocytes. *American Journal of Physiology* **25** E505–E508.
- Westermann J & Pabst R 1990 Lymphocyte subsets in the blood: a diagnostic window on the lymphoid system? *Immunology Today* **11** 406–410.

Received 29 October 1997

Revised manuscript received 13 February 1998

Accepted 6 May 1998