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ORIGINAL ARTICLE

The role of corticosterone in human hypothalamic–pituitary–adrenal axis feedback

Peter J. Raubenheimer*, Elizabeth A. Young†, Ruth Andrew* and Jonathan R. Seckl*

**Department of Endocrinology, University of Edinburgh, Centre for Cardiovascular Science, The Queen's Medical Research Institute, Edinburgh, UK and †Department of Psychiatry and Mental Health Research Institute, University of Michigan, Ann Arbor, MI, USA*

Summary

Objective In humans, the glucocorticoid corticosterone circulates in blood at 10–20-fold lower levels than cortisol, but is found in higher relative amounts in postmortem brain samples. Access of cortisol and corticosterone to the central nervous system may not be equal. Additionally, the relative affinities for the glucocorticoid and mineralocorticoid receptors differ, such that corticosterone may play a significant role in human brain function.

Design We measured cortisol and corticosterone levels in paired plasma and cerebrospinal fluid (CSF) samples. To test the relative potency of cortisol *vs.* corticosterone on hypothalamic–pituitary–adrenal (HPA) feedback, subjects underwent a three-phase, single-blind, randomized study assessing the postmetyrapone ACTH response over 3 h to an intravenous bolus of vehicle, cortisol or corticosterone (0·15 mg/kg and 0·04 mg/kg).

Participants Outpatients undergoing diagnostic lumbar puncture who were subsequently deemed to be free of disease. Feedback was tested in healthy male volunteers.

Measurements Plasma and CSF corticosterone to cortisol ratio was calculated and the ACTH response over time after the bolus glucocorticoid measured.

Results Plasma corticosterone : cortisol was 0·069 ± 0·007; CSF corticosterone : cortisol was 0·387 ± 0·050 ($P < 0·001$). Cortisol and corticosterone (0·15 mg/kg) suppressed ACTH *vs.* vehicle ($P = 0·002$); there was no difference between corticosterone and cortisol. The 0·04 mg/kg dose had no effect on ACTH despite supraphysiological plasma corticosterone levels.

Conclusions Corticosterone contributes almost 40% of total active glucocorticoids (cortisol and corticosterone) in the CSF. Significant effects on HPA axis suppression were only seen with supraphysiological levels of corticosterone, suggesting that corticosterone is not important in this model of nonstress-induced ACTH hypersecretion, in which the effect of cortisol predominates.

Correspondence: Dr Peter J. Raubenheimer, Edinburgh University, Centre for Cardiovascular Science, The Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK. Tel.: 44 131 2426773; Fax: 44 131 2426779; E-mail: praubenh@uctgsh1.uct.ac.za

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Introduction

Corticosterone is the predominant circulating glucocorticoid in rodents, but in humans it circulates at 10–20-fold lower concentrations than the principal human glucocorticoid, cortisol.[1–4] In humans, corticosterone is considered an intermediary in the formation of aldosterone; no specific role has been attributed to it. However, the multidrug resistance 1a (mdr1) P-glycoprotein membrane pump, which is highly expressed on the luminal surface of endothelial cells of the blood–brain barrier,[5] reduces access of the synthetic glucocorticoid dexamethasone and of physiological cortisol to the rodent brain *in vivo*.[6,7] Studies *in vitro* using cells transfected with the human MDR1 gene suggest a similar 'barrier' occurs in the human brain.[7] By contrast, corticosterone is not a substrate for mdr1a and readily passes the blood–brain barrier in rodents *in vivo*.[7] These data, alongside postmortem observations that the corticosterone : cortisol ratio is higher in brain tissue than in plasma,[7,8] have led to the suggestion that corticosterone might play a more prominent role than previously recognized in the effects of glucocorticoids upon the human brain.[7]

Glucocorticoid levels are tightly regulated by the activity of the hypothalamic–pituitary–adrenal (HPA) axis, itself subject to negative feedback control by glucocorticoids. Such central feedback is mediated by both mineralocorticoid (MR) and glucocorticoid receptors (GR) at several specific brain sites and, outside the blood–brain barrier, in the pituitary.[9] GR are lower affinity receptors that are distributed throughout the brain, but are particularly highly expressed in hypothalamic paraventricular nucleus neurones and corticotroph cells of the pituitary, while the higher affinity MR are mainly located in the limbic structures, in particular the hippocampus.[10–13] The relative roles of these two receptor subtypes in mediating corticosteroid feedback regulation of the HPA axis in different situations have not been fully resolved. GR are thought to play a major role in stress-responsive feedback because even at high levels of glucocorticoids there are significant numbers of 'available' unoccupied receptors.[12,14,15] MR have a much higher affinity for glucocorticoids so that they are

saturated at low glucocorticoid concentrations[14] and have accordingly been proposed to play a major role during the nadir of glucocorticoid production, in the evenings in humans, in maintaining inhibition of the HPA axis.[16] Nevertheless, Bradbury *et al.* in rats,[17] and Young *et al.* in humans,[18] using the MR antagonist spironolactone, showed that MR blockade affected HPA axis secretion at both the peak and the nadir of the circadian rhythm, even at times when GR was previously believed to be playing the major role in mediating glucocorticoid negative feedback.

The potential 'privileged' uptake of corticosterone in the brain might be expected to promote its receptor occupancy relative to cortisol. There is evidence that corticosterone has a higher affinity for human MR than cortisol[19,20] and thus it is possible that glucocorticoid-mediated effects on hippocampal functioning might reflect corticosterone acting through MR rather than cortisol.

We first aimed to measure cortisol and corticosterone in paired samples of plasma and cerebrospinal fluid (CSF) from living subjects to see if the previous results obtained from postmortem brain samples pertain *in vivo*. Thereafter we compared the effect of cortisol and corticosterone on ACTH suppression in a model of nonstress-induced ACTH hypersecretion after metyrapone administration. Metyrapone reduces cortisol and corticosterone production in the adrenal cortex by inhibiting their terminal biosynthetic enzyme 11β-hydroxylase (cyp11b1). Metyrapone also crosses the blood–brain barrier and blocks local generation of cortisol in the central nervous system, not only by inhibiting any 11β-hydroxylase in the brain but also by inhibiting 11β-hydroxysteroid dehydrogenase type 1, which regenerates both cortisol and corticosterone locally within neurones from inert 11-keto forms (cortisone, 11-dehydrocorticosterone) in the circulation.[21]

Methods

All studies were approved by the local research ethics committee and all subjects gave informed consent.

Experimental protocol

Study 1. We could not ethically justify doing a lumbar puncture on normal volunteers. CSF and venous blood were thus obtained within 5 min of each other from patients undergoing routine lumbar puncture as part of a work-up for possible neurological disease. All lumbar punctures were performed in the afternoon between 1430 and 1630 h. Of 23 sequential lumbar punctures performed, 11 were included for analysis in this study. Patients (mean age 39·2, range 23–70 years) were considered suitable for inclusion if no firm diagnosis of neurological disease affecting the central nervous system or blood–brain barrier was reached within 6 months following the lumbar puncture and if the CSF was normal with normal microscopy (with no white or red blood cells seen), cytology and biochemistry with normal CSF protein, albumin, immunoglobulin G (IgG) index and CSF : serum albumin ratio. No patients were on treatment with glucocorticoids for the preceding 6 months. Clinical indications for lumbar puncture were chronic headache (five patients), possible multiple sclerosis (four patients), polyneuropathy (one) and possible motor neurone disease (one).

Study 2. Ten healthy men of normal weight with no previous glucocorticoid exposure [mean age 32·5, range 23–45 years; mean body mass index (BMI) 22·9, range 20·7–25·2] underwent a three-phase, single-blind, randomized study assessing the postmetyrapone ACTH response over 3 h to an intravenous bolus of vehicle, cortisol (F) or corticosterone (B) (0·15 mg/kg). Six individuals underwent a further two phases using lower doses of F and B (0·04 mg/kg). Subjects were volunteers from the community in excellent physical and mental health as determined by an interview, a physical examination, routine laboratory tests and a structured questionnaire for psychiatric disorders.[22]

For each phase, at least a week apart, subjects took two separate oral doses of 750 mg metyrapone (Novartis, London, UK) at 0000 h and at 0600 h on the day of the study and attended the clinical research facility after an overnight fast. An intravenous catheter was inserted into an antecubital vein and subjects remained sedentary in bed for the period of the study. Basal plasma samples were collected between 0830 and 0900 h. Then, in random order, subjects were given an intravenous bolus (50 ml over 2 min) of (i) vehicle (0·9% saline); (ii) 0·04 mg/kg cortisol (hydrocortisone sodium succinate in 50 ml saline; Solu-Cortef, Pharmacia, Surrey, UK); (iii) 0·15 mg/kg cortisol; (iv) 0·04 mg/kg corticosterone (2% ethanolic solution; Calbiochem, EMD Biosciences Inc., San Diego, CA, USA); or (v) 0·15 mg/kg corticosterone. Blood samples were collected on ice for the next 3 h, plasma separated and aliquots stored at −80 °C until analysis.

Hormone assays

In study 1, CSF cortisol was measured with a sensitive salivary cortisol enzyme-linked immunosorbent assay (ELISA) kit (Salimetrics LLC, State College, PA, USA), plasma cortisol with an in-house radioimmunoassay and CSF and plasma corticosterone with an ELISA kit (Immunodiagnostic Systems Ltd, Boldon, UK). The cross-reactivity for corticosterone was 0·2% for the plasma cortisol assay and 0·1% for the CSF cortisol kit. The cross-reactivity of the corticosterone kit for cortisol, deoxycorticosterone, cortisone, 11-deoxycortisol and 17-OH progesterone was < 0·02%. In study 2, ACTH was measured by an ELISA kit (Biomerica, Newport Beach, CA, USA), plasma cortisol by ELISA, which does not cross-react with 11-deoxycortisol (DRG International Inc., East Mountainside, MG, USA), and corticosterone by ELISA as in study 1. The cross-reactivity of the plasma cortisol assay for corticosterone was 45% (but because plasma corticosterone is suppressed to < 20 nmol/l after metyrapone, this was considered negligible) and for deoxycorticosterone, 11-deoxycortisol, cortisone and 17-OH progesterone was < 2%. Importantly, the cross-reactivity of the plasma cortisol assay for hydrocortisone sodium succinate (Solu-cortef) was < 2%. Inter- and intra-assay coefficients of variation for all tests were < 8%.

Statistical analysis

For study 1, pairwise comparisons between groups were carried out by Student's paired *t*-test. For study 2, overall effects, summarized by the area under the curve (AUC) were assessed by two-way analysis of variance. Subsequent pairwise comparisons were made using

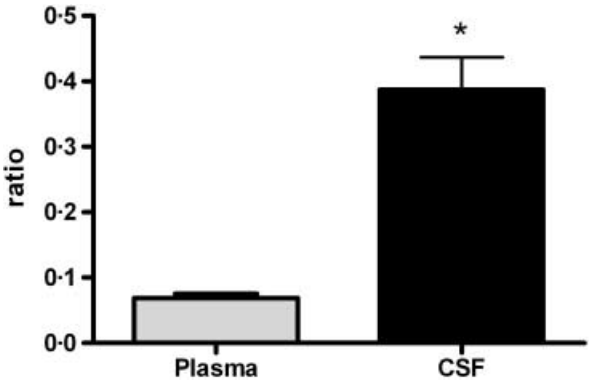

Fig. 1 The ratio of corticosterone over cortisol in human plasma and cerebrospinal fluid (CSF). Data are presented as mean ± SEM. *$P < 0{\cdot}001$.

Dunnett's test or Fisher's LSD test for comparison of equal dose infusions. Results are reported as mean ± SEM. $P < 0{\cdot}05$ was considered significant.

Results

Plasma to CSF ratios of corticosterone and cortisol

Plasma levels of cortisol were 830·4 ± 68·4 nmol/l (normal range 83–414 nmol/l), suggesting subjects were stressed at the time of sampling. Plasma corticosterone levels were 58·4 ± 9·2 nmol/l. The ratio of plasma corticosterone to cortisol was 0·069 ± 0·007, in keeping with previous studies.[2,7,23] CSF cortisol levels were 16·5 ± 1·7 nmol/l and CSF corticosterone levels were 6·3 ± 0·9 nmol/l. The ratio of corticosterone to cortisol in the CSF was 0·387 ± 0·050; this is 5·6-fold greater than in the simultaneously sampled plasma ($P < 0{\cdot}001$; Fig. 1).

ACTH suppression to cortisol and corticosterone

As expected, baseline plasma ACTH levels in the morning were elevated after metyrapone at 31·8 pmol/l (normal range 2–11·4 pmol/l). Baseline morning cortisol levels were low at 112·6 ± 9·8 nmol/l (normal range 138–634·8 nmol/l), confirming inhibition of glucocorticoid synthesis by metyrapone. After vehicle injection, plasma levels of ACTH, cortisol and corticosterone remained steady for the 180 min of the study (data not shown).

ACTH levels were significantly suppressed (Fig. 2) by both 0·15 mg/kg cortisol and 0·15 mg/kg corticosterone when compared with injection of vehicle (AUC of ACTH for cortisol 13 199·9 ± 2565·4 pg/ml/min *vs.* vehicle 26 439·5 ± 2621·7 pg/ml/min; $P = 0{\cdot}001$; AUC of ACTH after corticosterone 16 222·6 ± 2509·0 pg/ml/min *vs.* vehicle 26 439·5 ± 2621·7 pg/ml/min; $P = 0{\cdot}014$). There were, however, no differences in overall response between cortisol and corticosterone as measured by AUC of ACTH ($P = 0{\cdot}41$). Although the maximum percentage change in ACTH from baseline was greater after cortisol (90·4% *vs.* 71·7%, $P = 0{\cdot}002$), this difference appeared in the third hour after steroid bolus injection (AUC time 120–180 min, $P = 0{\cdot}007$; Fig. 2), which coincided with higher plasma levels of cortisol than corticosterone levels. ACTH levels after 0·04 mg/kg corticosterone or cortisol were not significantly different from vehicle or each other.

Plasma corticosterone and cortisol peaked within 5 min of the bolus dose, and then steadily declined over the next three study hours (Fig. 2). The concentrations of corticosterone achieved a significantly higher peak and declined faster to lower levels than those of cortisol. The half-life of cortisol was significantly greater than that of corticosterone ($T^1/_2$ of 0·15 mg/kg cortisol 91·9 ± 29·1 min *vs.* corticosterone 55·2 ± 17·5 min). Plasma levels of corticosterone and cortisol were thus not directly comparable across the study, most likely because of the delay in conversion of the inactive hydrocortisone sodium succinate (injected as Solu-cortef) to hydrocortisone that occurs in plasma[13] and/or because of differing pool sizes of the

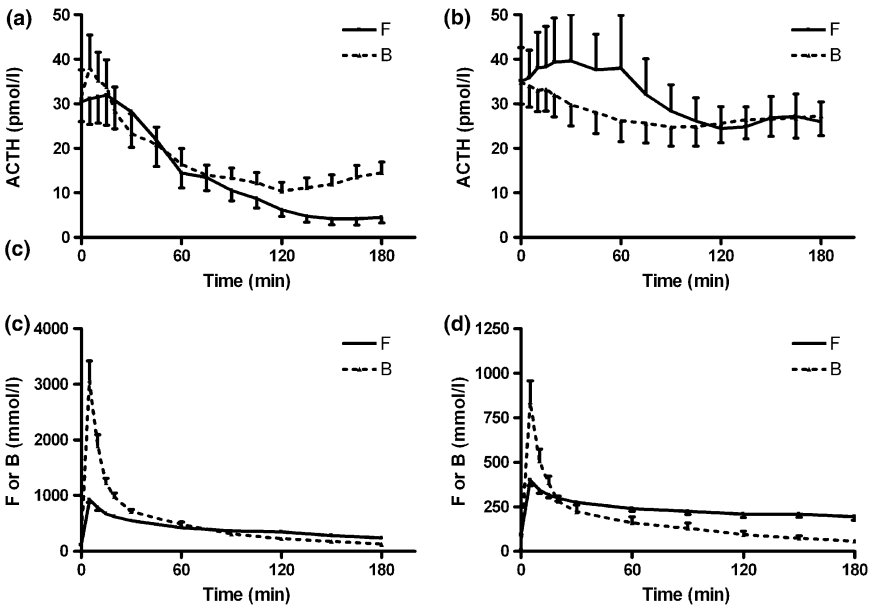

Fig. 2 Plasma ACTH levels (a,b) and corresponding cortisol (F) and corticosterone (B) levels (c,d) over time following a bolus dose of 0·15 mg/kg (a,c) or 0·04 mg/kg (b,d) cortisol (given as hydrocortisone sodium succinate) or corticosterone at time 0. Data are mean ± SEM and are presented as absolute values; analysis was by summary measures calculated from the curves.

different steroids and/or because of different elimination half-lives of cortisol and corticosterone.

Discussion

We have shown that the ratio of corticosterone to cortisol is significantly greater in human CSF than in plasma, with corticosterone contributing almost 40% of total active glucocorticoids (cortisol and corticosterone) in the CSF, whereas in plasma corticosterone is only 7% of the total. The ratio of corticosterone to cortisol obtained from the CSF *in vivo* is remarkably similar to that of Karssen *et al.*[7] who extracted glucocorticoids from human postmortem brain samples. This present analysis was performed at a time of stress due to lumbar puncture, which was reflected in the high plasma cortisol and corticosterone concentrations measured. It may be that the data may not reflect the situation at times of low basal circulating glucocorticoids, although there is no *a priori* reason to infer this. The relatively higher levels of corticosterone in CSF could be due to differential responses of cortisol and corticosterone to stress/ACTH. However, there is no evidence to suggest that cortisol and corticosterone respond differently to stress. Indeed, cortisol and corticosterone levels change in parallel both with the normal diurnal rhythm of glucocorticoid and in the increased secretion in depressive illness.[2,23] The plasma ratio for corticosterone to cortisol here is consistent with that reported in the literature from unstressed subjects.[2,7,23] It is possible that corticosterone is less bound to corticosteroid-binding globulin (CBG) than cortisol and thus more corticosterone is available for transport. However, the affinity of cortisol and corticosterone for CBG in the plasma is similar.[24] Finally, there may be differences in the rate of transport of the steroids into and/or clearance from the CSF. The relative rate of transport of cortisol and corticosterone into the CSF has not been studied, although, at least for cortisol, transport is very fast,[25] so this seems unlikely to account for proportionately higher corticosterone in CSF. It seems most plausible that proportionally higher corticosterone in CSF *in vivo* reflects a difference in rate of clearance of cortisol and corticosterone from the CSF. The most likely candidate mechanism is the blood–CSF barrier mdr1a P-glycoprotein membrane pump, as differences in bulk clearance from CSF, a passive process, seem intrinsically improbable. Both the blood–brain barrier and the blood–CSF barrier express MDR1; the blood–CSF difference measured is therefore also likely to be reflected by differential access of corticosterone across the blood–brain barrier. These data provide further support for the argument that the two steroids may not have equal access through the blood–brain barrier and suggest that the contribution played by corticosterone in glucocorticoid signalling in the brain is likely to be significant. Further mechanistic studies to investigate why this difference is present are clearly required. However, cortisol levels still exceed those of corticosterone in CSF, suggesting that it is the predominant signal even in the brain. Whether or not the greater affinity of corticosterone for MR tips the balance significantly in favour of this steroid under some circumstance remains uncertain.

We also report the first study on the action of corticosterone in human HPA feedback. We hypothesized that corticosterone would be more potent than cortisol in feedback. The results suggest that corticosterone does not play a predominant role in HPA feedback in this model of modest ACTH hypersecretion. Plasma levels of cortisol approached high physiological levels in the high-dose study and modest physiological levels in the low-dose study. A typical feedback response to a bolus dose of glucocorticoid was seen with ACTH levels starting to decline about 45 min after the bolus, with a maximal response after 2–3 h. Because of unexpected differences in plasma hormone concentrations after bolus injection, the study should be interpreted with caution. Peak plasma corticosterone levels at both doses were higher than cortisol levels and much higher than corticosterone levels expected even in response to severe stress. Despite this, the feedback by corticosterone was not more potent than cortisol. Indeed, there was no measurable ACTH suppression following the lower dose of corticosterone despite plasma levels high above physiological. Thus there is no evidence in favour of greater potency of corticosterone under the conditions tested.

This study examined acute effects of bolus doses designed to mimic an acute stress response. The relative contribution of pituitary *vs.* brain feedback in humans in response to endogenous glucocorticoids in this setting is not known, although a similar approach using bolus doses of glucocorticoids has been used in many studies testing central feedback,[26–28] with the available evidence suggesting that feedback to a bolus dose occurs at pituitary and central levels. This raises the question of whether cortisol and corticosterone are predominantly acting through the same or different glucocorticoid receptors centrally (GR or MR). Corticosterone has a lower affinity for GR than cortisol, and is less effective as an anti-inflammatory agent than cortisol by about a factor of three, suggesting lower GR potency.[29] By contrast, corticosterone has a higher affinity for MR than cortisol.[19,20] The lack of potency of corticosterone in HPA feedback suppression does not support the increase in levels of an MR preferential ligand in HPA axis suppression. This is perhaps unsurprising in the light of data from rodents suggesting that hippocampal MR are largely occupied by basal levels of glucocorticoids *in vivo*,[12,14,15] so large rises in steroid levels are anticipated to have little additional effect through this receptor. By contrast, GR are largely unoccupied under basal conditions, so rising levels of a preferential GR agonist such as cortisol may have greater effects in feedback suppression. This conjecture remains to be explored in humans *in vivo*.

This study does not support the contention that corticosterone is the predominant glucocorticoid affecting the brain in humans, at least in the situation of stress-type feedback. However, the possibility remains that corticosterone is important in central brain function because of the high concentrations found in CSF and brain and the reported relative affinity for MR over GR compared with cortisol. The proportional occupancy of MR and GR is also likely to differ across the range of circulating corticosteroid concentrations, such that we have not excluded the possibility that corticosterone is important in feedback in situations when signalling through MR may be dominant. Corticosterone may also play a role in more chronic effects upon the HPA axis or other CNS functions influenced by glucocorticoids, such as mood and cognitive function.

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References

1 Underwood, R.H. & Williams, G.H. (1972) The simultaneous measurement of aldosterone, cortisol, and corticosterone in human peripheral plasma by displacement analysis. *Journal of Laboratory and Clinical Medicine*, **79**, 848–862.

2 West, C.D., Mahajan, D.K., Chavre, V.J., Nabors, C.J. & Tyler, F.H. (1973) Simultaneous measurement of multiple plasma steroids by radioimmunoassay demonstrating episodic secretion. *Journal of Clinical Endocrinology and Metabolism*, **36**, 1230–1236.

3 Nishida, S., Matsumura, S., Horino, M., Oyama, H. & Tenku, A. (1977) The variations of plasma corticosterone/cortisol ratios following ACTH stimulation or dexamethasone administration in normal men. *Journal of Clinical Endocrinology and Metabolism*, **45**, 585–588.

4 Seckl, J.R., Campbell, J.C., Edwards, C.R., Christie, J.E., Whalley, L.J., Goodwin, G.M. & Fink, G. (1990) Diurnal variation of plasma corticosterone in depression. *Psychoneuroendocrinology*, **15**, 485–488.

5 Cordon-Cardo, C., O'Brien, J.P., Casals, D., Rittman-Grauer, L., Biedler, J.L., Melamed, M.R. & Bertino, J.R. (1989) Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood–brain barrier sites. *Proceedings of the National Academy of Sciences of the United States of America*, **86**, 695–698.

6 Meijer, O.C., de Lange, E.C.M., Breimer, D.D., de Boer, A.G., Workel, J.O. & de Kloet, E.R. (1998) Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. *Endocrinology*, **139**, 1789–1793.

7 Karssen, A.M., Meijer, O.C., van der Sandt, I.C.J., Lucassen, P.J., de Lange, E.C.M., de Boer, A.G. & de Kloet, E.R. (2001) Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse and human brain. *Endocrinology*, **142**, 2686–2694.

8 Brooksbank, B.W., Brammall, M.A. & Shaw, D.M. (1973) Estimation of cortisol, cortisone and corticosterone in cerebral cortex, hypothalamus and other regions of the human brain after natural death and after death by suicide. *Steroids and Lipids Research*, **4**, 162–183.

9 Keller-Wood, M.E. & Dallman, M.F. (1984) Corticosteroid inhibition of ACTH secretion. *Endocrine Reviews*, **5**, 1–24.

10 McEwen, B.S., Weiss, J.M. & Schwartz, L.S. (1968) Selective retention of corticosterone by limbic structures in rat brain. *Nature*, **220**, 911–912.

11 De Kloet, R., Wallach, G. & McEwen, B.S. (1975) Differences in corticosterone and dexamethasone binding to rat brain and pituitary. *Endocrinology*, **96**, 598–609.

12 Reul, J.M. & de Kloet, E.R. (1985) Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology*, **117**, 2505–2511.

13 Iwasaki, E. (1987) Hydrocortisone succinate and hydrocortisone simultaneously determined in plasma by reversed-phase liquid chromatography, and their pharmacokinetics in asthmatic children. *Clinical Chemistry*, **33**, 1412–1415.

14 Spencer, R.L., Young, E.A., Choo, P.H. & McEwen, B.S. (1990) Adrenal steroid type I and type II receptor binding: estimates of in vivo receptor number, occupancy, and activation with varying level of steroid. *Brain Research*, **514**, 37–48.

15 Spencer, R.L., Miller, A.H., Moday, H., Stein, M. & McEwen, B.S. (1993) Diurnal differences in basal and acute stress levels of type I and type II adrenal steroid receptor activation in neural and immune tissues. *Endocrinology*, **133**, 1941–1950.

16 de Kloet, E.R., Vreugdenhil, E., Oitzl, M.S. & Joels, M. (1998) Brain corticosteroid receptor balance in health and disease. *Endocrine Reviews*, **19**, 269–301.

17 Bradbury, M.J., Akana, S.F. & Dallman, M.F. (1994) Roles of type I and II corticosteroid receptors in regulation of basal activity in the hypothalamo–pituitary–adrenal axis during the diurnal trough and the peak: evidence for a nonadditive effect of combined receptor occupation. *Endocrinology*, **134**, 1286–1296.

18 Young, E.A., Lopez, J.F., Murphy-Weinberg, V., Watson, S.J. & Akil, H. (1998) The role of mineralocorticoid receptors in hypothalamic–pituitary–adrenal axis regulation in humans. *Journal of Clinical Endocrinology and Metabolism*, **83**, 3339–3345.

19 de Kloet, E.R., Veldhuis, H.D., Wagenaars, J.L. & Bergink, E.W. (1984) Relative binding affinity of steroids for the corticosterone receptor system in rat hippocampus. *Journal of Steroid Biochemistry*, **21**, 173–178.

20 Arriza, J.L., Weinberger, C., Cerelli, G., Glaser, T.M., Handelin, B.L., Housman, D.E. & Evans, R.M. (1987) Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science*, **237**, 268–275.

21 Raven, P.W., Checkley, S.A. & Taylor, N.F. (1995) Extra-adrenal effects of metyrapone include inhibition of the 11-oxoreductase activity of 11-beta-hydroxysteroid dehydrogenase: a model for 11-HSD I deficiency. *Clinical Endocrinology*, **43**, 637–644.

22 Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R. & Dunbar, G.C. (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*, **59** (Suppl. 20), 22–33.

23 Seckl, J.R., Campbell, J.C., Edwards, C.R.W., Christie, J.E., Whalley, L.J., Goodwin, G.M. & Fink, G. (1990) Diurnal variation of plasma corticosterone in depression. *Psychoneuroendocrinology*, **15**, 485–488.

24 Stroupe, S.D., Harding, G.B., Forsthoefel, M.W. & Westphal, U. (1978) Kinetic and equilibrium studies on steroid interaction with human corticosteroid-binding globulin. *Biochemistry*, **17**, 177–182.

25 Martensz, N.D., Herbert, J. & Stacey, P.M. (1983) Factors regulating levels of cortisol in cerebrospinal fluid of monkeys during acute and chronic hypercortisolemia. *Neuroendocrinology*, **36**, 39–48.

26 Fehm, H.L., Voigt, K.H., Kummer, G., Lang, R. & Pfeiffer, E.F. (1979) Differential and integral corticosteroid feedback effects on ACTH secretion in hypoadrenocorticism. *Journal of Clinical Investigation*, **63**, 247–253.

27 Wolf, O.T., Convit, A., de Leon, M.J., Caraos, C. & Qadri, S.F. (2002) Basal hypothalamo–pituitary–adrenal axis activity and corticotropin feedback in young and older men: relationships to magnetic resonance imaging-derived hippocampus and cingulate gyrus volumes. *Neuroendocrinology*, **75**, 241–249.

28 Boscaro, M., Paoletta, A., Scarpa, E., Barzon, L., Fusaro, P., Fallo, F. & Sonino, N. (1998) Age-related changes in glucocorticoid fast feedback inhibition of adrenocorticotropin in man. *Journal of Clinical Endocrinology and Metabolism*, **83**, 1380–1383.

29 Berger, T.S., Parandoosh, Z., Perry, B.W. & Stein, R.B. (1992) Interaction of glucocorticoid analogues with the human glucocorticoid receptor. *Journal of Steroid Biochemistry and Molecular Biology*, **41**, 733–738.