Lack of Fpr2/Fpr3 receptors alters the structure and function of pituitary corticortrophs

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ABSTRACT

Background: The N-formyl peptide receptor (FPR) family of G-protein-coupled receptors, originally identified to recognise N-formylated bacterial peptides, has in more recent times been shown to bind Annexin-1 (ANXA1). ANXA1 is a signalling molecule well demonstrated to mediate two key glucocorticoid effects in the anterior pituitary: inhibition of adrenocorticotropic hormone (ACTH) release from corticotrophs, and regulation of granule exocytosis. Whilst previous reverse transscriptase polymerase chain reaction (RT-PCR) studies have detected Fpr2, Fpr3, Fpr-N and Fpr-NC receptors mRNA in murine pituitary tissue, the specific member(s) of the FPR family involved in ANXA1 signalling are unclear. In this study, we investigated whether glucocorticoid inhibitory feedback is impaired in Fpr2/Fpr3 double knockout (DKO) mice.

Methods: Anterior pituitary tissue from wild-type and Fpr2/Fpr3 DKO male mice was fixed and examined (i) by electron microscopy to determine corticotroph size, granule morphology and rough endoplasmic reticulum (rER) expansion, and (ii) by immunocytochemistry to determine corticotroph density. Plasma ACTH and corticosterone concentrations were determined by radiomunnoassay.

Results: In Fpr2/Fpr3 DKO mice, corticotrophs exhibited a significant (P<0.01) increase in rER expansion and a significant (P<0.05) decrease in granule density (%) suggesting increased ACTH synthesis and secretion, together with elevated levels of plasma ACTH (P<0.05) and corticosterone (P<0.05). Fpr2/Fpr3 DKO mice exhibited a significant (P<0.01) increase in corticotroph cell density.

Conclusion: These data suggest that there is a loss of ANXA1-mediated glucocorticoid action in Fpr2/Fpr3 DKO mice, indicating that Fpr2 and/or Fpr3 may function as ANXA1 receptor(s) in the anterior pituitary.

Introduction

Corticotrophs are endocrine cells of the anterior pituitary at the centre of HPA axis regulation, responsible for adrenocorticotropic hormone (ACTH) synthesis and release, the glucocorticoid-releasing hormone (CRH) and the inhibitory control of plasma glucocorticoids (GCs). A substantial body of evidence supports the role of Annexin 1 (ANXA1) as a paracrine mediator of glucocorticoid (GC) feedback in the anterior pituitary. Experimental observations are consistent with the proposal that GC-induced ANXA1 exocytosis from folliculostellate cells is associated with inhibition of ACTH secretion from adjacent corticotrophs.

The antiapoptotic effects of glucocorticoids are thought to be at least partially mediated by ANXA1. Indeed, in ANXA1 knockout male mice a four-fold increase in the number of corticotrophs has been noted with no associated change in hypothalamic CRH expression, suggesting that ANXA1 directly influences corticotroph proliferation in the anterior pituitary.

The FPR family of G-protein-coupled receptors consists of three human genes designated FPR1, FPR2, and FPR2L on chromosome 19 in conserved synteny with the mouse FPR gene cluster on chromosome 17. Genomic screening in mice using cDNA probes for human FPRs has revealed eight distinct mouse genes: Fpr1, Fpr2, Fpr3, Frpr-r3, Fpr-r4, Fpr-r5, Fpr-r6 and Fpr-r7. Murine Fpr2 and Fpr3 are highly homologous (92%) receptors transcribed from the same DNA template strand and share structural and pharmacological properties with the human FPR2 receptor. The identity of the precise FPR subtype(s) involved in glucocorticoid feedback in the anterior pituitary is currently unknown.

Hypothesis: ANXA1-mediated glucocorticoid feedback in the anterior pituitary is impaired in Fpr2/Fpr3 double knockout (DKO) mice.

Methods

Animals

Do/23 double knockout mice were produced by targeted deletion of Fpr2 and Fpr3 genes in embryonic stem (ES) cells as first described by Dunton et al. All wild-type and Fpr2/Fpr3 DKO mice were sacrificed at 3 months of age. Trunk blood was collected for assay of plasma ACTH and corticosterone.

Detection of Fpr2 by Western blot analysis

Fpr2 was detected by overnight incubation of proteins extracted from pituitary tissue by sonication with a rabbit anti-Fpr2 polyclonal antibody 1:3000 dilution (Abcam, Cambridge, UK) then anti-rabbit IgG conjugated to horseradish peroxidase 1:5000 dilution (Sigma-Aldrich, Co, Poole, UK).

Quantitative Electron Microscopy

In order to identify the corticotrophs in the pituitary immunogold labelling for ACTH was used. The sections were viewed with a V1010 transmission electron microscope (JEOL, Peabody, MA, USA). Six micrographs of corticotroph per animal were taken at a magnification of 40000x for analysis of cell size, granule morphology and rough endoplasmic reticulum (rER) expansion. Negatives were scanned into Adobe Photoshop (version 5.5) and transferred to Axiovision (version 4.5) software for image analysis. The total granule area and granule density (%) calculations were carried out using Microsoft Office Excel 2007 (version 12.0). Expansion of rER was assessed visually from electron micrographs and quantified on a scale of 0-3, 0 being no expansion and 3 the most expansion. In all cases, the analysis was blind to the sample code.

Immunofluorescence

Sections of anterior pituitary tissue were examined using a TCS confocal microscope (Leica Corp, Microsystems, Wetzlar, Germany). Corticosterone density for wild-type and Fpr2/Fpr3 DKO mice was quantified by counting ACTH immunopositive cells per 100 µm x 100 µm of section (determined by Axiovision 4.2) for eight different sections.

Plasma ACTH and corticosterone measurement

Plasma ACTH and corticosterone concentrations were determined by radioimmunoassay.

Statistical analysis

All data were expressed as mean ± s.e.m. Statistical analysis was performed using GraphPad Prism (version 4). Statistical significance was determined using a one-way ANOVA with post hoc analysis performed using the Bonferroni test. In all cases, differences were considered significant if P<0.05.

Results

Figure 1: ANXA1 expression in the anterior pituitary of WT mice

Figure 2: ANXA1 expression in the anterior pituitary of WT mice

Figure 3: In the Fpr2/Fpr3 DKO mice, an overall increase in corticotroph cell size was measured although the increase in total cell area (SA) and cytoplasmic area (CA) was not statistically significant. In Fpr2/Fpr3 DKO mice, corticotrophs had significantly (P<0.05) larger nuclei (N).

Figure 4: In Fpr2/Fpr3 DKO mice, corticotrophs had a reduced total granule area although this change was not significantly different to wild-type corticotrophs (48). Granule density (%) was significantly (P<0.001) reduced in Fpr2/Fpr3 DKO mice compared with wild-type (48). In Fpr2/Fpr3 DKO corticotrophs, granule diameter was not affected (46) however the rough endoplasmic reticulum (rER) was significantly (P<0.01) expanded (40).

Figure 5: In Fpr2/Fpr3 DKO mice, plasma levels of ACTH (SA) and corticosterone (SB) were significantly (P<0.05) increased for both (each).

Figure 6: The results from fluorescence microscopy indicate that there is a significant (P<0.01) increase in corticotroph density in Fpr2/Fpr3 DKO mice (4C).

Figure 7: Representative electron micrographs

In the present study, we have demonstrated that Fpr2 and/or Fpr3 deficiency is associated with increased ACTH synthesis and secretion and a dramatic increase in corticotroph density consistent with the hypothesis that glucocorticoid (GC) inhibitory feedback is impaired in Fpr2/Fpr3 double knockout (DKO) male mice.

The reduction in total granule area and granule density (%) observed in Fpr2/Fpr3 DKO corticotrophs suggests that in the absence of GC inhibition, ACTH granules are released causing depletion of the intracellular stores. The diameter of granule area and granule density (%) observed in ANXA1 knockout mice (48) is unaffected (P<0.001) by glucocorticoids. In Fpr2/Fpr3 DKO mice, the four-fold increase in corticotroph density compared with wild-type mice may additionally reflect the function of ANXA1 in pro-apoptotic signalling.

In this study, Western blot analysis also indicated that Fpr3 is expressed in the hypothalamus. Therefore, an alternative explanation for corticotroph population expansion in Fpr2/Fpr3 DKO mice may be loss of ANXA1-mediated GC action on the hypothalamus, including GC suppression of CRH mRNA to regulate the mitogenic effects of CRH on the anterior pituitary. Hence, the aim of future studies will be to dissect the relative contribution of GC feedback on the hypothalamic changes in corticotroph structure and function observed in Fpr2/Fpr3 DKO mice.

Additionally, in this study we have demonstrated Fpr3 expression in the folliculostellate (FS) TdT/TGF cell line, which to our knowledge has not been shown previously. This may present an autocrine feedback loop whereby externalised ANXA1 binds Fpr3 on FS cells to regulate its own release.