Targeting cognitive impairments in anxiety disorders: transcriptional actions of glucocorticoid receptor modulators.

Chinedu T Udeh[1], Eleanor J Waite[1], Fiona J Thomson[2] and Stafford L Lightman[1]

[1]Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, Dorothy Hodgkin building, Bristol, UK; [2]Merck, Sharp and Dohme, Neuroscience target ID/ validation team, West Point, USA.

Background and Rationale

➢ HPA axis activation leads to pulsatile production and release of glucocorticoid (GC) hormones .

➢The HPA axis pathway is characterised by the release of Corticotrophin releasing factor (CRF) from the PVN of the hypothalamus. CRF is carried in the portal circulation to the pituitary where it acts on its receptors on corticotroph cells to stimulate the release of ACTH. ACTH is released into the blood and acts on its receptors in the adrenal glands to induce synthesis and release of glucocorticoids (cortisol in man and corticosterone in rodents) [figure 1A].

➢GCs are fundamental for survival, and act via its receptors (GR) on target organs to regulate various physiological processes [Figure 1B], and a key mechanism underlying GC actions in the brain is via receptor-mediated regulation of target gene expression. However, the normalcy of this process is thought to be dysregulated in conditions like major psychotic depression, and the use of GR antagonists may be beneficial in altering the deleterious effects of GCs in such disease states.

➢Here, we examine the actions of a novel non-steroidal GR antagonist, termed Org A and the steroidal GR antagonist RU-486 (Mifepristone), on the transcriptional response to GCs in the mouse brain.

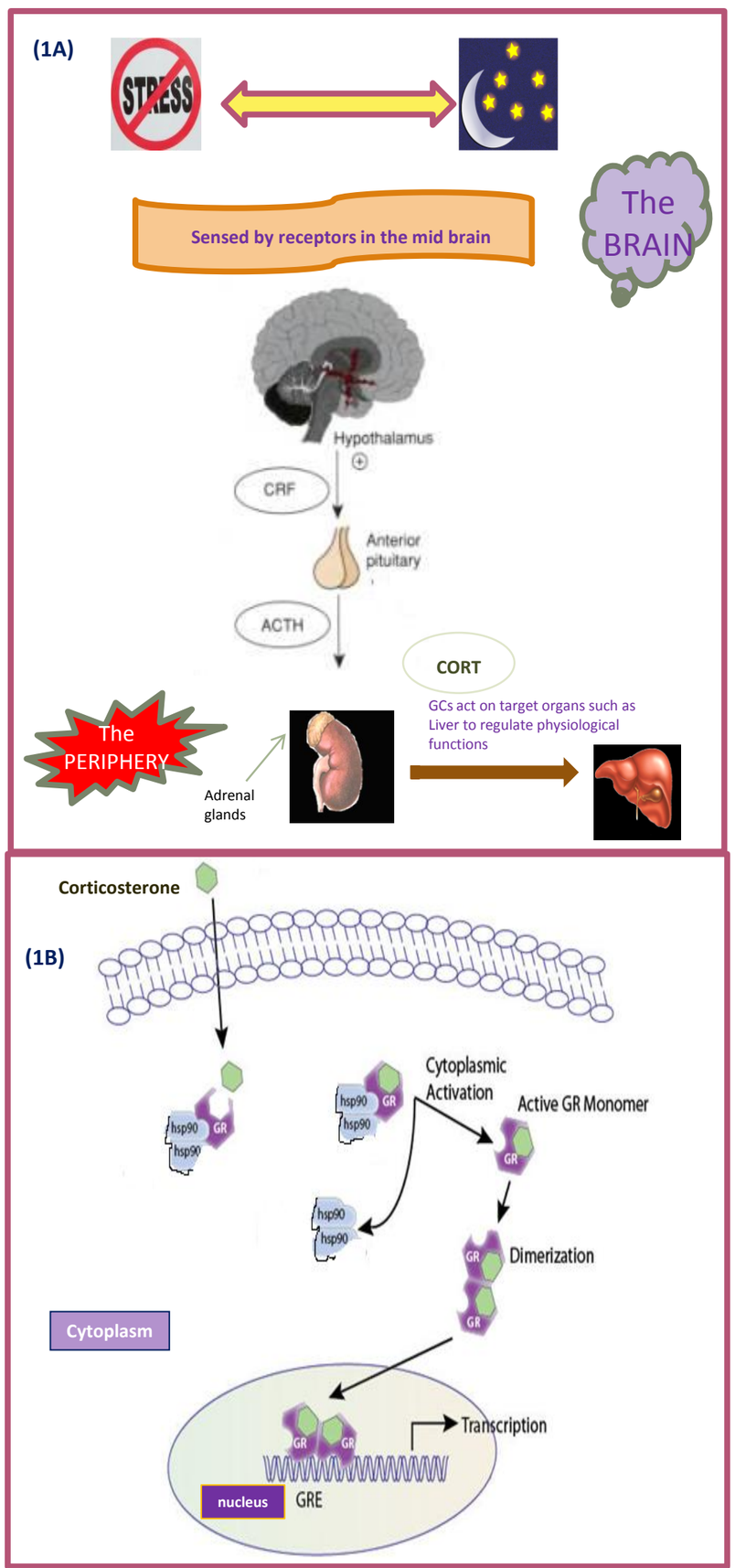

Figure 1: Actions of Glucocorticoid (GC) (A) Synthesis and release of GCs by HPA axis activation. (B) Simple representation of the Glucocorticoid receptor signalling pathway.

Aim of Study

To investigate the effects of Org A and RU-486 on the transcriptional response to exogenous corticosterone (cort) mediated increase in circulating GCs in the mouse hippocampus, amygdala and prefrontal cortex.

Methods

C57BL6/J adult mice were used in this study. All animals underwent bilateral adrenalectomy. Animals were treated as follows:

Pilot study: s/c injections of vehicle (DMSO +Mulgofen 1:5), followed by an i/p bolus of corticosterone (3mg/kg) which mimics the plasma corticosterone profile observed in an acute stress response. Animals in the baseline group received s/c vehicle and i/p saline injections. Trunk blood was collected at 0, 30, 60, 120 and 180 mins following i/p injections. Plasma corticosterone levels were measured by RIA. [figure 2].

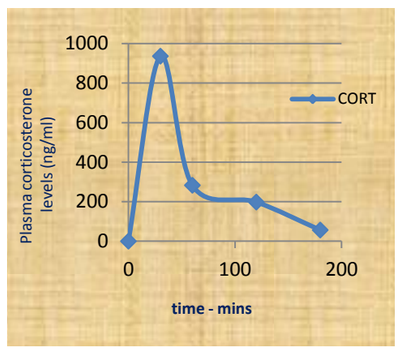

Figure 2. Temporal profile of plasma corticosterone levels in mice following corticosterone administration..

Group 1- nuclear translocation study: animals were treated with s/c injections of vehicle (DMSO +Mulgofen 1:5) and received 3mg/kg ip cort or i/p saline injections 30 minutes later.
Group 2 – gene expression study: animals were administered s/c vehicle (DMSO +Mulgofen 1:5), 20mg/kg Org A or 20mg/kg RU-486; followed by i/p injections of either 3mg/kg cort or saline 60 minutes later.

Hippocampi, amygdala and pre-frontal cortex were dissected out and collected on dry ice, at times corresponding to the circadian nadir (9-11am) of the animal's diurnal rhythm (see figure 3).

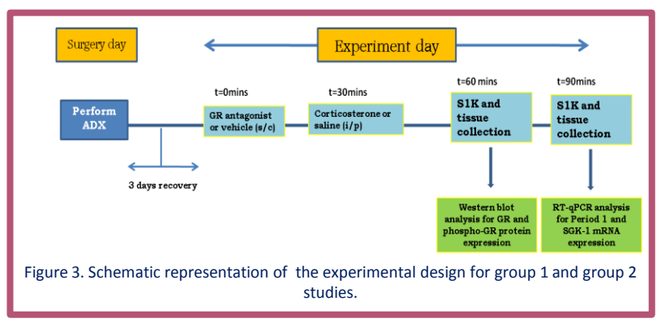

Figure 3. Schematic representation of the experimental design for group 1 and group 2 studies.

➢To show nuclear translocation, as well as protein localization, Western blot analysis was conducted on nuclear fractions prepared from hippocampi, amygdala and prefrontal cortex [figure 3]. Blots were probed for GR or phosphorylated GR protein.
➢To examine regulatory actions of the GR antagonists, Org A and RU-486 on expression of GC responsive genes in our study conditions, we conducted real time quantitative PCR (RT-qPCR) assays to look at period-1 (per-1) and serum and glucocorticoid inducible kinase (sgk-1) mRNA expression, which are well characterised GC inducible genes, and have been implicated in the pathophysiology of depression [figure 3]. .

Results

Effect of glucocorticoid on GR activation in ADX mice

Administration of corticosterone resulted in elevated plasma corticosterone levels peaking at 30 mins after the CORT injection [Figure 2]. This triggered an increase in GR translocation into the nucleus as high protein levels were seen in response to the GC signal. Marked increase in GR activation, evident by hyperphosphorylation at the Ser-845 residue of the receptor protein was also observed between experimental groups in all the tissues studied, namely hippocampus [figure 4A], amygdala [figure 4B] and prefrontal cortex [figure 4C].

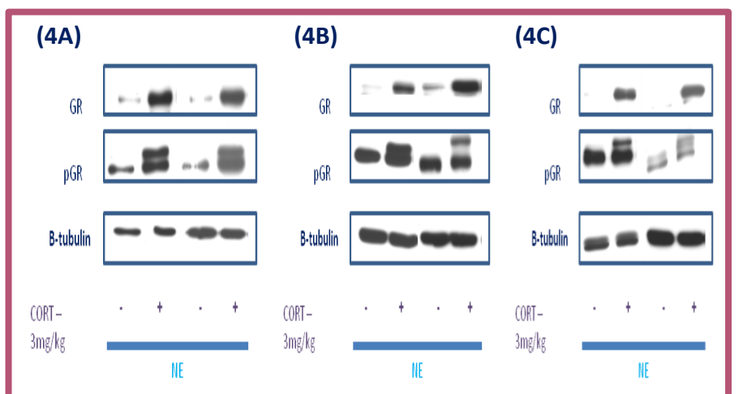

Figure 4: Western blot depicting sub cellular localization and activation states of GR in hippocampus (4A), amygdala (4B) and prefrontal cortex (4C) collected 30mins following i/p injections of Cort. Beta-tubulin used as loading control for nuclear extracts (NE).

Effect of glucocorticoid receptor antagonists on gene transcription regulated by GR in ADX mice

Period-1 and sgk-1 mRNA expression was measured at 90 minutes post administration of vehicle, Org A or RU-486, and 60 mins following i/p CORT and compared to vehicle + saline treated animals. CORT administration resulted in significantly increased sgk-1 mRNA levels in the hippocampus, amgdala and prefrontal cortex. Similar GC responsiveness was also observed in hippocampal period-1 expression, but not in amygdala or prefrontal cortex.

In the hippocampus, both Org A and RU-486 potentiated the effect of CORT on sgk-1 induction, whilst abolishing the GC mediated increase in period-1 expression. Org A and RU-486 augmented the CORT induced rise in sgk-1 levels in the amygdala, however neither GR antagonist had any discernable effect on period-1 transcription levels.
Interestingly, in the prefrontal cortex, whist Org A had no transcriptional effect on sgk-1 expression, decreased sgk-1 mRNA levels were seen in the RU-486 treated group. No effects on period 1 mRNA induction was noted with either GR antagonist in this region.

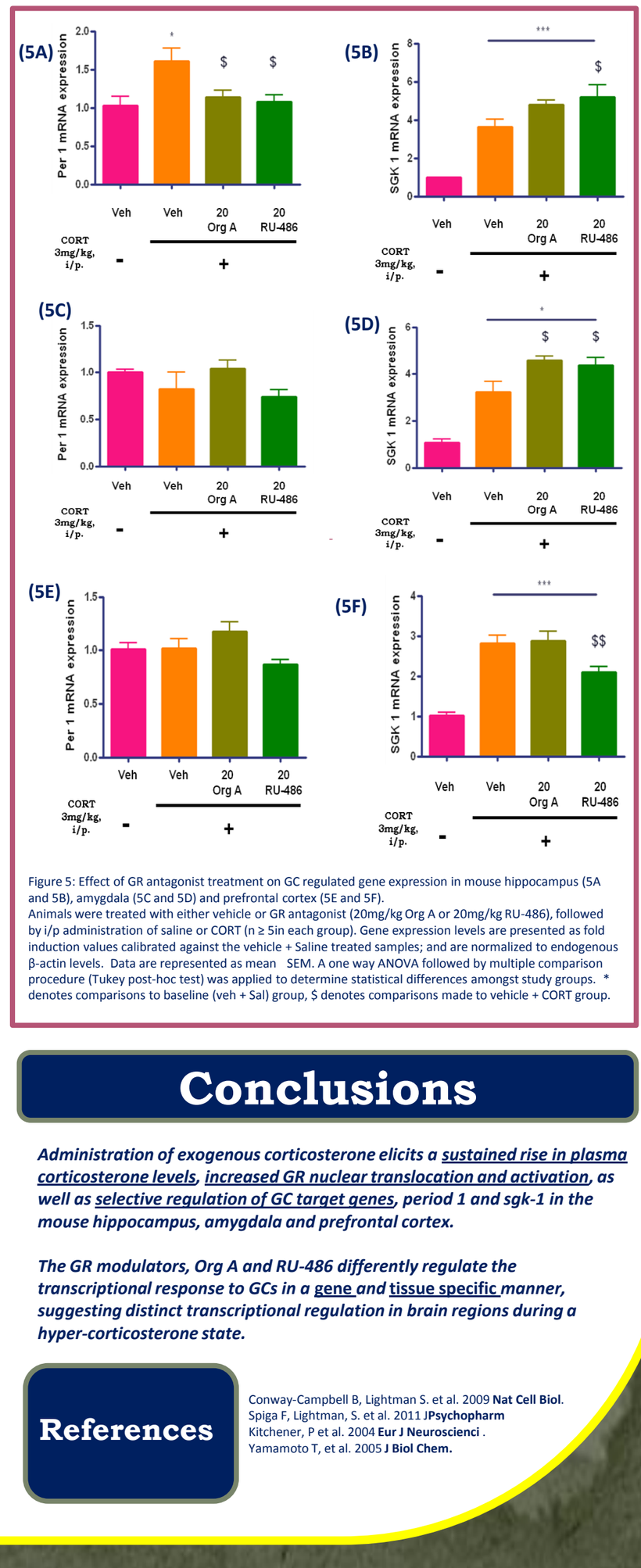

Figure 5: Effect of GR antagonist treatment on GC regulated gene expression in mouse hippocampus (5A and 5B), amygdala (5C and 5D) and prefrontal cortex (5E and 5F).
Animals were treated with either vehicle or GR antagonist (20mg/kg Org A or 20mg/kg RU-486), followed by i/p administration of saline or CORT (n ≥ 5in each group). Gene expression levels are presented as fold induction values calibrated against the vehicle + Saline treated samples; and are normalized to endogenous β-actin levels. Data are represented as mean SEM. A one way ANOVA followed by multiple comparison procedure (Tukey post-hoc test) was applied to determine statistical differences amongst study groups. * denotes comparisons to baseline (veh + Sal) group, $ denotes comparisons made to vehicle + CORT group.

ORG A

	SGK-1	PER-1
HC	↑	↓
AMY	↑	✖
PFC	✖	✖

RU-486

	SGK-1	PER-1
HC	↑	↓
AMY	↑	✖
PFC	↓	✖

Key: ↑ = up regulated; ↓ = down regulated; ✖ = no effect

Figure 6: Differential effects of Org A and RU-486 on sgk-1 and per-1 mRNA induction in the hippocampus, amygdala and prefrontal cortex.

Conclusions

Administration of exogenous corticosterone elicits a sustained rise in plasma corticosterone levels, increased GR nuclear translocation and activation, as well as selective regulation of GC target genes, period 1 and sgk-1 in the mouse hippocampus, amygdala and prefrontal cortex.

The GR modulators, Org A and RU-486 differently regulate the transcriptional response to GCs in a gene and tissue specific manner, suggesting distinct transcriptional regulation in brain regions during a hyper-corticosterone state.

References

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Acknowledgements

Dr Becky L Conway-Campbell and Miss Annie J Pellatt

'FJT' is an employee of Merck + Co. Org A was provided by Merck + Co"

Email to: chinedu.udeh@bristol.ac.uk