# Investigation of the molecular mechanisms underlying the anti-inflammatory properties of 5α-tetrahydrocorticosterone

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## BACKGROUND

- Glucocorticoids (GCs) are widely used for the treatment of chronic inflammatory conditions (e.g. rheumatoid arthritis). Their use is accompanied by serious side effects such as the metabolic syndrome and hypertension.
- A major scientific effort is in place to find so called "dissociated compounds" that retain the therapeutic anti-inflammatory properties, while lacking the ability to promote unwanted effects.
- GCs signal intra-cellularly by many diverse processes, which can be dimer dependent or independent, and which differ between pathways regulating inflammation and metabolism.
- Recent work has revealed that a metabolite of corticosterone (B), namely 5α-tetrahydrocorticosterone (5α-THB), has anti-inflammatory properties *in vivo* without inducing adverse metabolic effects.

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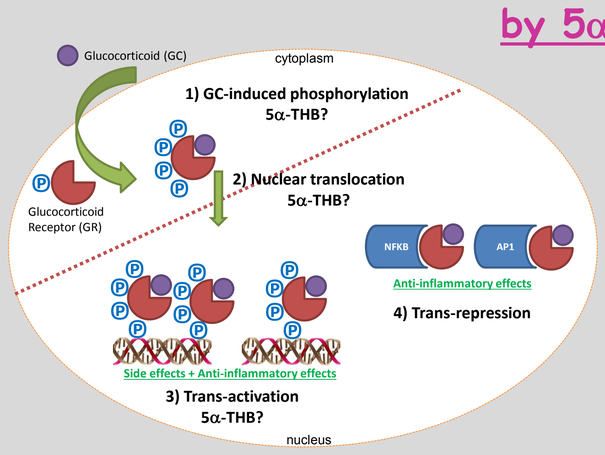
## Question: what molecular signalling pathways are influenced by 5α-THB?




**Schematic representation of glucocorticoid-dependent molecular processes taking placing inside the cell. Three of them, phosphorylation, translocation and trans-activation, are the subject of this study and will be investigated in response to 5α-tetrahydrocorticosterone (5α-THB).**

## HYPOTHESIS

**GR bound with 5α-THB induces some but not all signalling pathways typical of glucocorticoid hormones**

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## METHODS

- The ability of 5α-THB to induce phosphorylation of GR serine 211 ($^{ser211}$GR), a key residue in determining GR activity, was studied by Western blotting in the human pulmonary cell line A549, incubated with steroids for 1h.
- To determine mobility of ligand-bound GR, localisation of green fluorescent protein tagged-rat GR (GR-GFP) transfected in the human kidney cell line HEK293 was monitored by fluorescence microscopy.
- Ligand ability to induce transcription was studied in HEK293 cells by gene reporter induction assay, where the reporter luciferase was under control of either a promoter requiring GR dimers (MMTV) or GR multimers (PNMT) to drive gene expression. Transfected cells were incubated with steroids for 48h.
- Effects of steroids on mRNA levels of the endogenous GC-responsive metabolic gene tyrosine aminotransferase (*Tat*) were investigated in the mouse hepatoma cell line BWTG3, naturally expressing GR, by qPCR after treatment for 4 to 24 hours.

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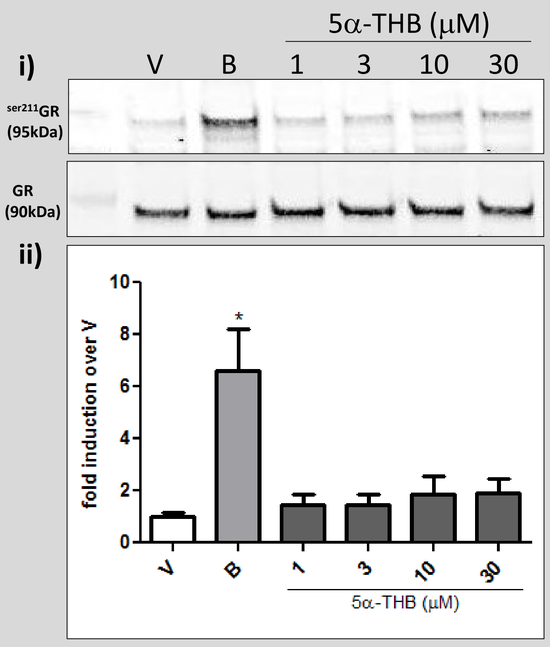
## RESULTS

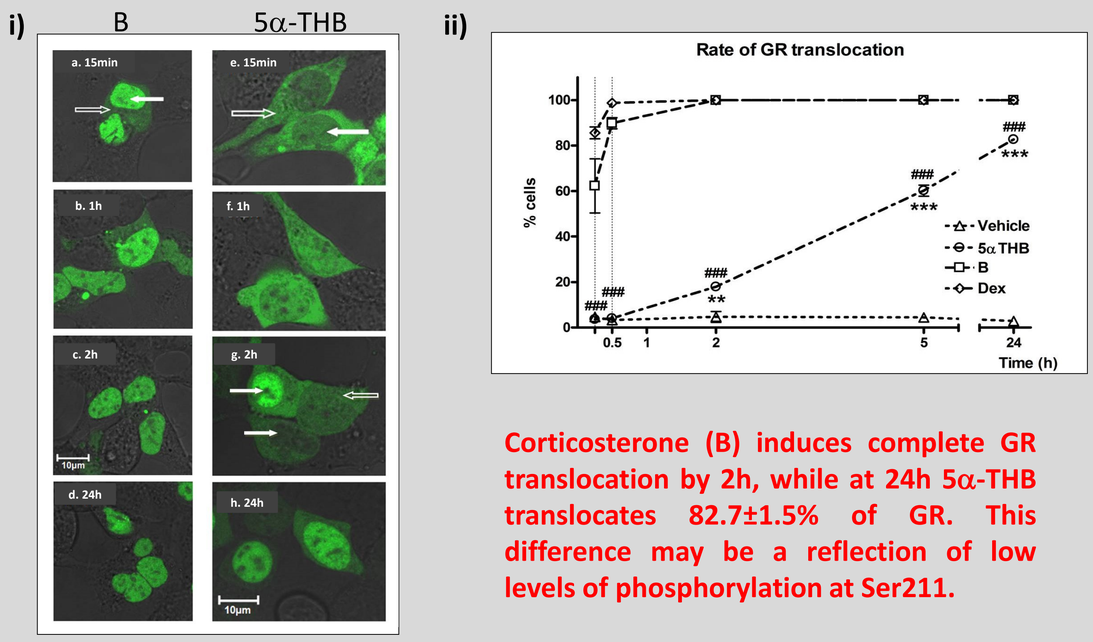
### 1) 5α-THB does not phosphorylate $^{Ser211}$GR




**Following ligand binding GR becomes hyper-phosphorylated at Ser211. Phosphorylation of this amino-acid has been linked with translocation of GR to the nucleus and trans-activation of endogenous genes and reporters such as MMTV- and PNMT-Luc.**

**Figure 1** (i) Western blot analysis of $^{Ser211}$GR and GR in lung adenocarcinoma cell line A549 cells treated either with corticosterone (B, 1μM), 5α-tetrahydrocorticosterone (5α-THB) or vehicle (V) for 1 hour. (ii) Quantitative analysis of GR phosphorylation was calculated as ratio $^{Ser211}$GR/GR and represented as induction over vehicle. Comparisons were performed by one-way ANOVA with Dunnett's post-hoc tests. Data are mean ± SEM; * p < 0.05 vs vehicle group; n=4.

### 2) 5α-THB translocates GR slowly to the nucleus




**Corticosterone (B) induces complete GR translocation by 2h, while at 24h 5α-THB translocates 82.7±1.5% of GR. This difference may be a reflection of low levels of phosphorylation at Ser211.**

**Figure 2** (i) Representative images of time course of GFP-GR translocation to the nucleus induced by (a-d) corticosterone (B) and (e-h) 5α-tetrahydrocorticosterone (5α-THB), both 1μM. Open arrow represents cytoplasm; full arrow represents nucleus. (ii) Quantification of GFP-GR translocation induced by either 1μM B, Dexamethasone (Dex,) or 5α-THB. Data are mean ± SEM; n=3; **p<0.01, ***p<0.001 versus vehicle; ###p<0.001 versus B, analysed for the effect of 5α-THB by two-way repeated measures ANOVA (one factor repetition) with Holm-Sidak post-hoc tests.

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### 3) 5α-THB does not promote activation of GC-responsive reporters

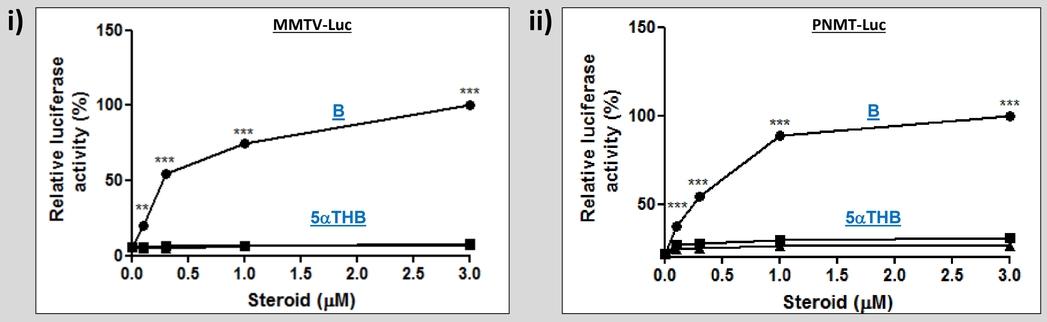


**Figure 3** Promoter induction analysis for the GR dimer-dependent reporter MMTV-Luc (i) and the GR multimer-dependent reporter PNMT-Luc (ii), transiently transfected into HEK293 cells, in response to treatment with different concentrations (0.01-3μM) of either corticosterone (B) or 5α-tetrahydrocorticosterone (5α-THB). Comparisons were made using two-way ANOVA with Bonferroni's post-hoc tests. ** p<0.01 *** p<0.001 vs vehicle; Data are represented as percentage of induction compared to the maximal response achieved with B, 3μM. Data are mean ± SEM, n=4.

### 4) 5α-THB does not increase mRNA levels of the metabolic gene *Tat* … but suppresses the action of B

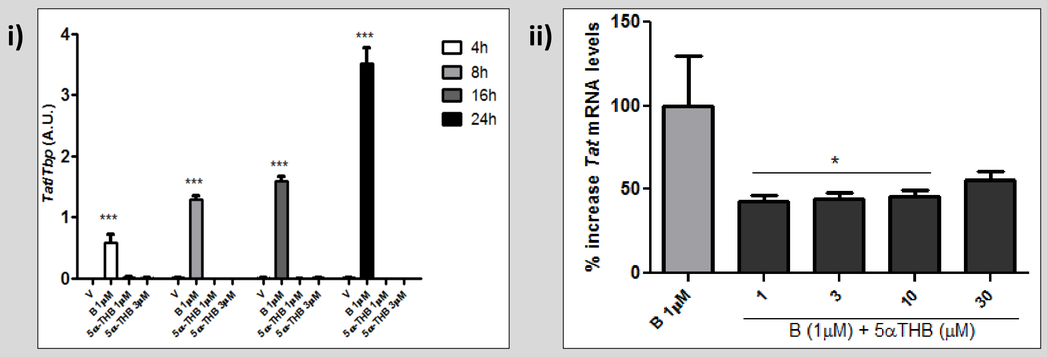


**Figure 4**. Quantification by real time PCR of tyrosine aminotransferase (*Tat*) mRNA levels in response to treatment with vehicle (V), corticosterone (B) or 5α-tetrahydrocorticosterone (5α-THB) of BWTG3 hepatoma cells. (i) Time-dependent variations of mRNA levels and (ii) mRNA levels after 16h treatment represented as percentage increase. B is considered as giving the maximal percentage increase (100%). Data are mean ± SEM; n=3. Comparisons were made using one-way ANOVA with Bonferroni's post-hoc test. *** = p<0.001 vs V; * = p<0.05 vs B. A.U. = arbitrary units; TBP = TATA box binding protein.

**Tyrosine aminotransferase (*Tat*) is expressed in the liver where it is involved in gluconeogenesis. It is up-regulated by glucocorticoids and it is thought to be responsible for some of the side effects of these hormones. The absence of induction by 5α-THB confirms the good metabolic profile found in previous *in vivo* experiments. The fact that the action of B is decreased by the presence of 5α-THB suggests that 5α-THB may work as a partial agonist.**

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## CONCLUSIONS

- **5α-THB does not phosphorylate GR at Ser211 and fails to induce GR dimer/multimer-dependent pathways activated by classical glucocorticoids. This agrees with our previous *in vivo* findings that this steroid does not influence metabolism unlike conventional glucocorticoids.**
- **However 5α-THB can bind GR and induce translocation, behaving like a partial agonist to suppress the action of corticosterone.**
- **In liver, where it is formed mainly in vivo, 5α-THB may therefore attenuate the effects of endogenous glucocorticoids on metabolic processes.**