**Compound A, a Selective Glucocorticoid Receptor Agonist Displaying Dissociation of Transactivation from Transrepression**

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Current anti-inflammatory drugs, such as glucocorticoids (GC), have severe side effects. These side effects are mostly mediated through transactivation of particular genes, whereas the anti-inflammatory effects are mainly mediated through repression of pro-inflammatory genes. Therefore, dissociated glucocorticoids, which effectively transrepress via the glucocorticoid receptor (GR), while showing little transactivation potential, are the current focus of the search for safer anti-inflammatory drugs with fewer side effects.

Compound A (CpdA), a synthetic analogue of a non-steroidal compound from *S. tuberculatiformis*, causes contraception in rats and prolonged gestation in sheep. We have previously shown that CpdA mediates its biological actions at a molecular level via interaction with a variety of steroid-binding proteins[1,2], decreases corticosteroid-binding globulin (CBG) levels, and increases free corticosterone levels in rats[3]. The next step was to investigate interaction with the GR.

Although the liganded GR is involved in a plethora of interactions in the process of eliciting its physiological response, our study focuses on specific steps in the pathway of liganded GR. These steps were selected on the basis that there is some evidence in the literature that the activity is likely to be an indicator of pharmacological characteristics. We investigate and compare the molecular mechanism of action of CpdA with that of medroxyprogesterone (MPA), reported to be a dissociated GR ligand[4], and dexamethasone (DEX), a potent GR agonist.

Whole cell competitive binding studies indicate that although CpdA binds endogenous rat GR with a significantly lower affinity than either DEX or MPA, it does compete with 3H-DEX for rat GR with nanomolar affinity. In addition, CpdA binds reversibly to the rGR and influences association of 3H-Dex, but has no effect on dissociation. Both promoter-reporter studies and RT-PCR of endogenous genes indicate that CpdA transrepresses, but does transactivate GC-responsive genes *in vitro*. We show that CpdA behaves as a compound with complete dissociation of transactivation and transrepressive GC action in our systems, in contrast to MPA, that only displays partial dissociative activity. Further studies with co-transfected GR and RU486, a GR antagonist establish that the repressive action of CpdA is mediated by the GR. In addition, we show that CpdA causes nuclear translocation of the GR like DEX and MPA, but appears to elicit an activated GR conformation that differs from that elicited by DEX, as assessed by the production of different protected GR fragments in limited proteolysis and by creating a GR that does not appear to dimerise.

Thus, CpdA may represent a novel, non-steroidal, dissociated GC, worthy of further investigation for therapeutic applications. Furthermore, this atypical

GR ligand could shed light on the fundamental mechanisms underlying the ligand-selective regulation of gene expression by the GR.

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