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GLUCOCORTICOID HEDGEHOG AGONISTS IN NEUROGENESIS

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Abstract

The process of neurogenesis in mammals, which is prolific and widespread at birth, gradually slows with aging and in humans becomes restricted to areas including the cerebellum and hippocampus. It has been reported that exposure to glucocorticoids can impair neurogenesis in both adults and children. Glucocorticoids are known to bind with high affinity to intracellular receptors. Glucocorticoid blood levels are normally regulated by environmental stresses, but because of their clinical utility, exogenous glucocorticoids are frequently administered in drug formulations. Consequently, concerns have arisen about the consequences of glucocorticoid use on neurogenesis and health, especially in the pediatric population. In this article we will review recent findings that a select number of related glucocorticoids also bind the Hedgehog pathway receptor Smoothed. We will discuss their pharmacology and also a most surprising result; that this select group of compounds, which includes FDA approved drugs, unlike typical glucocorticoids such as dexamethasone, stimulate stem cell growth and thus enhance neurogenesis.

Introduction:

The ability of stem cell neurons in the central nervous system of mammals to divide is not entirely lost with aging. It has recently been shown in humans that stem cell neuronal populations remain active in the hippocampus, suggesting that memory and behavior may remain plastic throughout adulthood and responsive to external stressors such as drugs (Schoenfeld and Gould, 2011). Mood disorders are prevalent in the general adult population and Lithium, a commonly used drug to treat depression, appears to enhance adult hippocampus neurogenesis by preventing GSK3 β activity up-regulation mediated by nuclear glucocorticoid receptors (Boku et al., 2010). The ability of glucocorticoids to inhibit neurogenesis has become a clinical concern in children where negative effects on brain development are apparent in infants exposed to dexamethasone (Heine and Rowitch, 2009), and the chronic use of steroids has increased in the pediatric population for diseases like asthma. In general, children exposed to glucocorticoids over extended periods have an increased association with cognitive deficits and a reduction in brain size (Damsted et al., 2011).

Seven transmembrane receptors on stem cells regulate many of the growth and patterning pathways for organogenesis and tissue repair. For example, Frizzled receptors signal through GSK3 β in the gastrointestinal tract to control levels of beta-catenin, whose elevation is associated with tumors (Doucas et al., 2005). Similarly, abnormally elevated signaling of the

seven transmembrane receptor Smoothed (Smo), a Hedgehog pathway regulator normally suppressed by Patched, underlies development of medulloblastoma in the cerebellum (Corcoran and Scott, 2001). While endogenous Wnt agonists have been identified for Frizzled, no endogenous agonist has been discovered for Smo. A limited number of synthetic Smo agonists, however, have been discovered by screening compound libraries and testing their activity in the presence of the Smo antagonist cyclopamine (Chen et al., 2002). For our program in stem cell and regenerative medicine, we developed a similar strategy to identify Smoothed ligands using a novel β -arrestin-GFP translocation assay (Wang et al., 2010), but rather than screen combinatorial libraries we have concentrated our efforts on FDA approved drugs. In one such screen we surprisingly identified a restricted series of glucocorticoids with Smoothed activity (Wang et al., 2010). Moreover, these select compounds in contrast to glucocorticoids in general stimulate rather than inhibit the growth of cerebellar neuronal progenitor cells.

Select Glucocorticoids as Smo Agonists: Potential Effects for Neurogenesis:

The Hedgehog signaling pathway, mediated by Smo, is critical for embryonic patterning, stem cell growth, and central nervous system development (Ingham and McMahon, 2001; Marti and Bovolenta, 2002; Palma et al., 2005; Ruiz i Altaba et al., 2002). Therapeutic activation of Hedgehog signaling by Smo agonists has been proposed for restoring tissue function in the peripheral and central nervous systems for retinal injury, Parkinson's disease, spinal cord damage, and neuronal degeneration (Bambakidis et al., 2009; Tsuboi and Shults, 2002; Wan et al., 2007; Li et al., 2008). The cerebellum contains one of the most abundant populations of neurons in the brain, derived from a stem cell population of cerebellar granular cell precursors (GCPs), and the *in vivo* expansion of these granule precursor cells is dependent on Hedgehog/Smo pathway signaling (Wechsler-Reya and Scott, 1999). For the mouse cerebellum in the first postnatal week, purkinje cell secreted sonic hedgehog (Shh) ligand stimulates GCP proliferation in the outer layer of the cerebellar surface (external granular layer). GCPs then exit the proliferative phase of the cell cycle, differentiate into granule neurons, and migrate inward into the internal granule layer (Figure 1).

Using a high-throughput screen of cells containing Smo and β -arrestin-GFP reporter that binds activated Smo, we identified four glucocorticoids - halcinonide, fluticasone propionate, clobetasol propionate, and fluocinonide as Smo agonists (Wang et al., 2010). Similar to some other known Smo agonists, SAG and purmorphamine, these glucocorticoids bind Smo, promote Smo internalization, and activate Gli. Consistent with Smo agonist activity, these select set of glucocorticoids stimulate the proliferation of GCPs alone and synergistically, and such stimulation was not affected by the glucocorticoid nuclear receptor antagonist Mifepristone (RU-486). In contrast, the glucocorticoid receptor (GR) agonist dexamethasone *inhibited* Shh-activated GCP proliferation. Additionally, GCP treatment with Shh, Purmorphamine, SAG, halcinonide, clobetasol propionate, and fluticasone propionate (but not the weak Smo agonist fluocinonide) inhibit caspase 3 degradation and block GCP apoptosis by increasing endogenous cyclin D2 protein expression. No such growth enabling

responses were observed in GCPs treated with cortisone, dexamethasone, prednisolone, and corticosterone.

As expected the four glucocorticoid Smo agonists and dexamethasone had similar effects in activating GR in a GR-GFP nuclear translocation assay, yet dexamethasone produced an opposite response to the glucocorticoid Smo agonists on GCP proliferation and apoptosis. Taken together these data suggest that the GCP proliferative response is due directly to activation of Smo and is independent of glucocorticoid nuclear receptor signaling. The ability of some glucocorticoid drugs, particularly FDA approved compounds, to activate Hedgehog signaling has profound implications for pediatric therapeutics, stress related disorders, and regenerative medicine. These drugs have well-known safety profiles and are well characterized, so that their application in new therapeutic areas can be evaluated much more expeditiously than unapproved compounds isolated in screens of compound libraries.

Mechanism of Action

Glucocorticoid steroids are a standard of care in many clinical settings including rheumatic disorders, asthma, inflammation and cancer (Schimmer 2006). An excellent summary of the medicinal chemistry of steroid hormones and therapeutically related compounds and a review of the x-ray crystal structures of steroidal compounds provide keen insights into the medicinal chemistry of steroidal glucocorticoids (Proteau, 2004; Duax et al., 1988). Glucocorticoid steroids bind the glucocorticoid receptor, a member of the nuclear receptor superfamily (Figure 2). Upon binding to GR, the cytoplasmic form of the receptor moves to the nucleus and activates the transcription and repression of multiple gene products involved in metabolism and inflammation. In particular, glucocorticoids such as dexamethasone acting through the GR receptor inhibit cell growth in GCPs. Independent of GR signaling, select glucocorticoids such as halcinonide bind to the Smo receptor, activate Hedgehog signaling, and stimulate cell growth in GCPs (Figure 2). While these select glucocorticoids can be both GR agonists and Smo agonists, the Smo agonist effects appear to be the more dominant in GCPs.

Structure-Activity Relationships of Smo Agonist Glucocorticoids

Subsequent screening of focused chemical libraries in the β -Arrestin2-GFP translocation assay has increased the number of glucocorticoid derivatives identified with Smo agonist activity (Table 1). These efforts have helped define key structure activity relationships (SAR) for Hedgehog agonist activity while also identifying a number of structurally-related glucocorticoid derivatives without Smo agonist activity (Table 2).

The most dominant trends noted for Smo agonist activity to date are the nature of the substituents at C-17, a hydroxyl group in the beta configuration at C-11, and a fluorine atom on the alpha face at C-9. Of these trends, a review of the inactive compounds suggests the most dominant feature for Smo agonist activity may be the nature of the substitution at the C17 position in conjunction with a hydroxyl group in the beta position at C-11. Though each of the active Smo agonists contains a hydroxyl group in the beta configuration at C-11, there are a number of glucocorticoid derivatives with an 11-beta hydroxyl group that are inactive

Smo agonists. These inactive Smo agonists often possess a C-17 substituent that is more hydrophilic, less branched and generally smaller. Some of these compounds also have a fluorine atom in the C-9 alpha position. Based on these observations, a working model was created to guide SAR exploration and to aid our understanding of the structural features of glucocorticoid derivatives that drive potency and activation of the hedgehog pathway (Figure 3).

Conclusion

Although glucocorticoids for the most part are known to inhibit neurogenesis as nuclear receptor agonists, we identified a subgroup of them that bind Smoothed and are capable of stimulating neuron proliferation. This unusual finding resulted from a high throughput drug screening approach, was confirmed by secondary cell based assays, and formed the basis for a program of SAR analysis to characterize the chemical determinants underlying the binding. This subgroup of steroids present us with an unprecedented opportunity to jump start clinical trials assessing the therapeutic benefits of potentiating Hedgehog signaling, because these same glucocorticoid drugs have been used clinically by tens of millions and their safety profiles are well know. Areas in which they may provide immediate benefit and could be tested include Parkinson's disease, spinal cord injury, myocardial repair post infarction, and wound healing.

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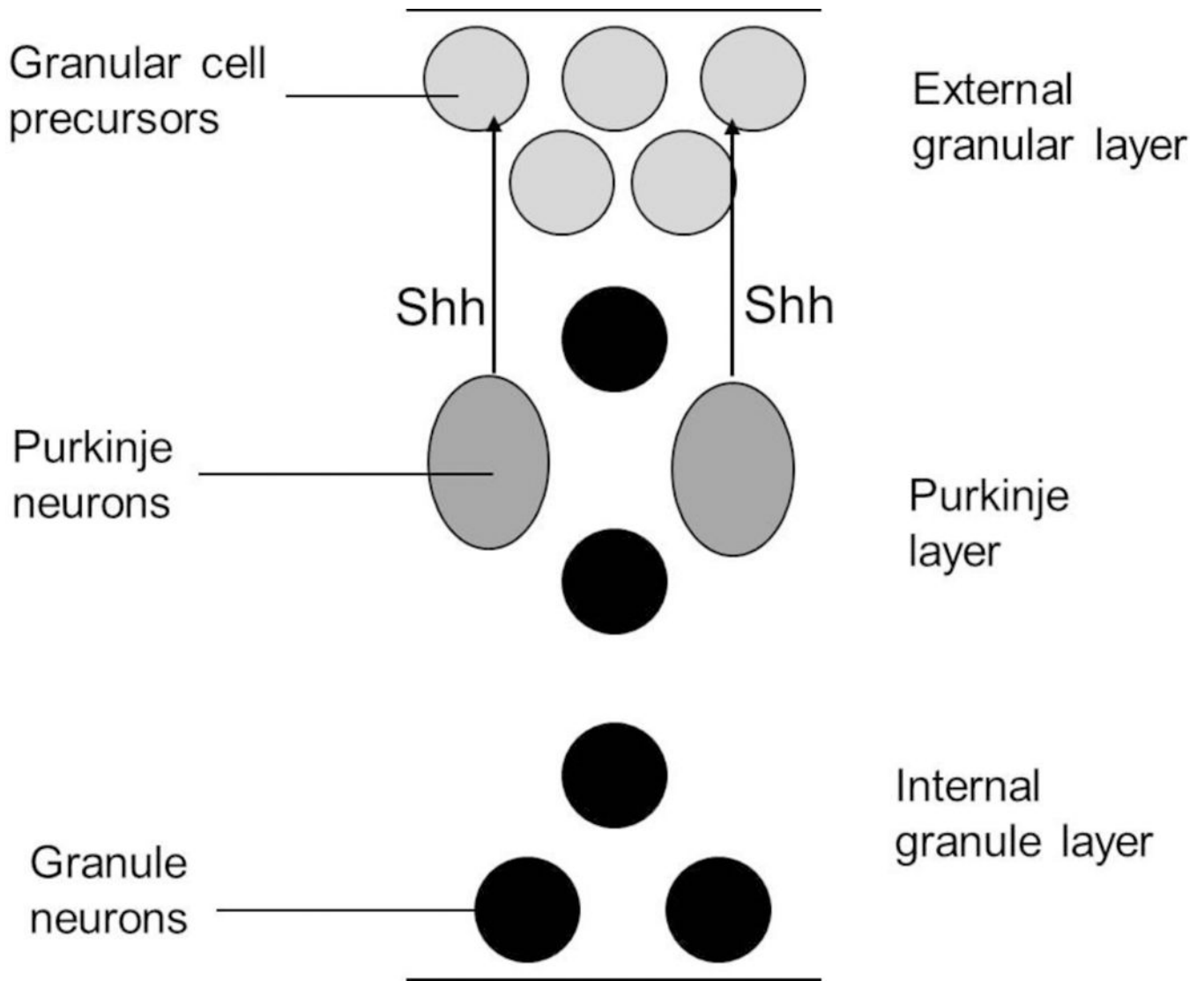


Figure 1. Primary neuronal granule cell precursor (GCP) proliferation in the cerebellum is Hedgehog signaling dependent.

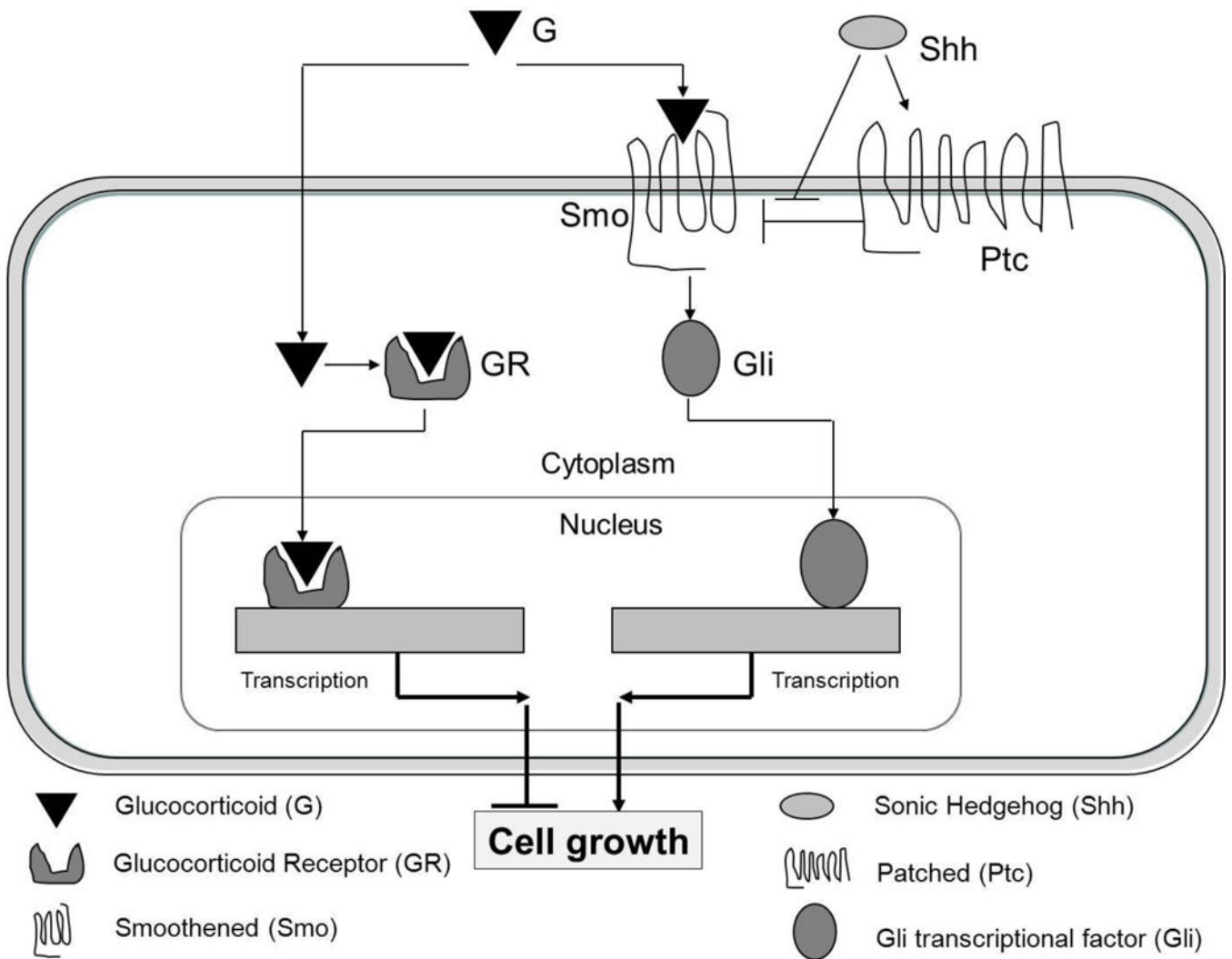


Figure 2. Select glucocorticoids affect cell growth through both the glucocorticoid receptor and the Smo receptor.

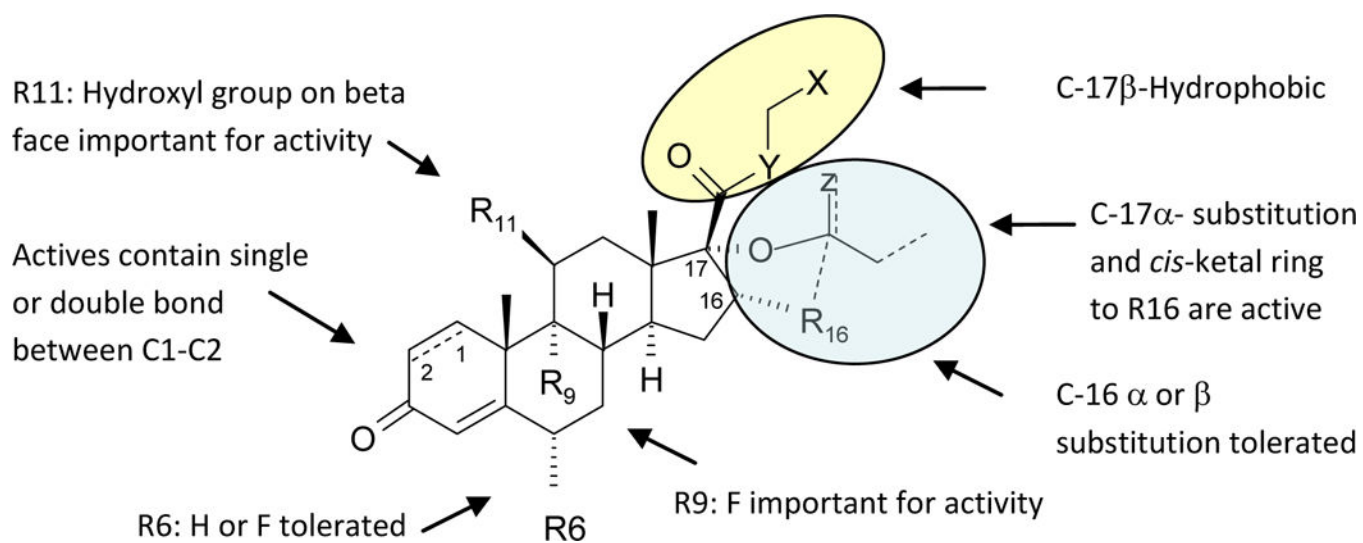


Figure 3.
Working model of glucocorticoid Smo agonist activity.

Table 1.Summary of glucocorticoid derivatives with Smo agonist activity in the β -arrestin2-GFP translocation assay

Compound	EC ₅₀ in β -arrestin2-GFP translocation assay (uM)
Fluticasone Propionate ^a	0.099
Halcinonide ^a	1.1
Clobetasol Propionate ^a	1.5
Fluocinonide ^a	>5
Fluticasone	0.1
Triamcinolone Acetonide	0.4
Halobetasol Propionate	0.8
Fluocinolone Acetonide	1.5
Amcinonide	3

^aWang *et al.*,2010

