Steroid Hormones and Receptors
STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Mechanism of Action of Irilone as a Potentiator of Progesterone Receptor Signaling

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Progesterone signaling and its proper regulation is important for reproductive function. When progesterone signaling is dysregulated, gynecological diseases can occur, for example endometriosis, uterine fibroids, and endometrial cancer. While these diseases are treated with progestin therapy, progestins can bind to multiple steroid receptors, exerting side effects of weight gain, immunosuppression, cardiovascular disease, and stroke. Discovering an alternative progestin that is selective for the progesterone receptor (PR) is ideal. One potential source of such an alternative is botanical dietary supplements, which have become increasingly popular among consumers with sales reaching $9.6 billion in 2019. Although botanical supplements are popular, the chemical structures and biological action of botanical supplements would benefit from deeper scientific investigation. Studies of *Trifolium pratense L.* (red clover), primarily used for the treatment of menopausal symptoms, identified phytoestrogen compounds as the chemicals that mitigate those symptoms. Interestingly, irilone, identified from red clover, potentiated progesterone signaling via a progesterone response element luciferase (PRE/Luc) assay. Potentiation is when a compound has no activity by itself but when combined with another molecule, i.e. progesterone, that compound enhances PR activity. Prior to irilone, a natural compound with the ability to potentiate progesterone signaling had not been previously reported. The purpose of this study was to determine the mechanism of action of irilone. We hypothesized that irilone was potentiating PR by blocking PR degradation and by altering PR post-translational modifications. Irilone was found to potentiate 5 nM P4 using a PRE-luciferase assay in both T47D and Ishikawa PR expressing cells. Since PR is a downstream target gene of ER, we investigated if irilone also had ER activity. Irilone increased expression of an ERE-luciferase reporter gene. Next, we investigated if irilone could stabilize PR degradation and if irilone altered PR phosphorylation via western blot. Irilone was found to increase PR protein levels, but when ER was blocked, this was mitigated. In the presence of P4, irilone did not increase phosphorylation of serine 294 on PR. Future studies will determine if irilone is altering sumoylation of PR, and if irilone can potentiate PR signaling *in vivo*. Determining how irilone is potentiating progesterone will help us understand PR biology and could be an effective treatment for gynecological diseases by enhancing endogenous progesterone action.

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Melanocortin 2 Receptor Antagonists in Canine Cushing's Disease: In Vitro Studies

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Melanocortin 2 receptor antagonists in canine Cushing's disease: in vitro studies
Cushing's disease (CD), caused by an ACTH-secreting pituitary adenoma, is one of the most common endocrinopathies in dogs. The current medical treatment options involve adrenocortical steroid synthesis inhibitors, but a selective targeted approach to block ACTH receptor at its receptor would be much more attractive. The objective of this study was to preclinically investigate the effect of MC2R antagonists on adrenocortical hormone production, cell viability, and mRNA expression of steroidogenic enzymes in canine primary adrenocortical cell cultures from adrenal glands of healthy dogs. Three different MC2R antagonists were used: CRN.1, CRN.2, and CRN.4. Canine primary adrenocortical cell cultures (n = 8) were incubated with 50 nM ACTH for 24h, to mimic CD. Thereafter, 10 nM (IC50) and 2 μM (maximal concentration) of CRN.1, CRN.2, and CRN.4 were added. The two concentrations were established based on preliminary studies. After 24 hours of incubation, adrenocortical hormone concentrations were measured in the culture medium using liquid chromatography-mass spectrometry. RNA was isolated from the cells using the RNeasy Microkit (Qiagen) for subsequent real-time quantitative PCR analysis. Cell viability was assessed after 24 hours of incubation using alamarBlue™ Cell Viability Reagent. All CRN compounds effectively inhibited cortisol concentrations, while leaving aldosterone concentrations unaffected. In incubations with a maximal concentration of the three compounds, cortisol concentration decreased to undetectable levels. The mRNA expression levels of steroidogenic enzymes StAR, CYP11A1, CYP17A1, HSD3B2, CYP21, and CYP11B were significantly inhibited in most conditions when compared to the ACTH-stimulated control. The mRNA expression of melanocortin 2 receptor accessory protein (MRAP) was suppressed as well. Cell viability was not affected by CNR.1 or CNR.4, but was slightly inhibited by CRN.2. In summary, canine adrenocortical cell culture is a useful model system for drug testing. Incubation with MC2R antagonists demonstrated the potential of CNR.1 and CNR.4 as new treatment options for CD. Future in vivo studies in dogs with spontaneous CD are indicated.

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One Hormone for Two Receptors: Exploring Glucocorticoid Actions Mediated by the Glucocorticoid and Mineralocorticoid Receptors

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Glucocorticoids are indispensable for mediating the response to stress, energy demands, development, and limiting inflammation. Once in the cell, these hormones exert