Differential regulation of iNOS expression and function by dexamethasone, fluticasone and hydrocortisone in vascular smooth muscle cells.

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Introduction: The up-regulation of the inducible nitric oxide synthase (iNOS) and nitric oxide production (NO) have been implicated in inflammatory pathologies (1). Previous research revealed that dexamethasone (*Dex*; a non-selective steroid, which acts on glucocorticoids, mineralocorticoids, androgen, and progesterone receptors) inhibited iNOS expression and hence NO production (2). However, the underlying mechanism(s) that mediate these effects of *Dex* remain unclear. The aim of this study therefore was to further investigate the mechanism(s) of action of *Dex* in relation to iNOS expression and function in cultured vascular smooth muscle cells. Furthermore, the actions of *Dex* were compared to those of hydrocortisone (*Hdc*; another non-selective steroid) and fluticasone (*Flu*; a highly selective glucocorticoid) in the presence of selective receptor antagonists to distinguish between the glucocorticoid and non-glucocorticoid actions of *Dex*. Additionally, we have initiated studies to investigate novel signaling pathways that may mediate the actions of *Dex* and have focused on protein kinase R (PKR), an effector molecule for interferons and a key intracellular pathway for Toll-like receptor activation, growth factors, and a diverse range of other cellular stresses.

Methods: All experiments were conducted using primary cultures of rat aortic smooth muscle cells (RASMCs). Concentration dependent effect of *Dex* (0.1-10μM) on NO production and iNOS expression were determined to corroborate previous findings. Studies with *Hdc* (0.01-10μM) and *Flu* (0.1-30 nM) have also been initiated, examining their effects on NO production and iNOS expression in cells activated with lipopolysaccharide (LPS; 100μg ml^{-1}) and interferon-gamma (IFN-γ 100 IU ml^{-1}). To identify the receptor pathways involved, cells were pre-treated with either 10μM mifepristone (*Mfp*; glucocorticoids and progesterone receptor antagonist) or 100 nM-10 μM Eplerenone (*Eprn*; selective mineralocorticoids receptor antagonist) for 1 h prior to incubation with *Dex*, *Hdc* or *Flu*. To establish whether PKR is involved in iNOS induction and linked to the effects of *Dex*, *Hdc* and/or *Flu*, concentration dependent effect of PKR inhibitor, C16, (0.03-1 μM) on NO production and iNOS expression were examined. Nitrite levels were quantified by the Greiss assay and iNOS expression determined by western blotting 24 h after activation of cells (2). . One-way ANOVA followed by Dunnett's test was utilised to seek statistical differences using GraphPad Prism version 5.0.

Results: Both *Dex* and *Hdc* virtually abolished iNOS expression and NO production in a concentration dependant manner. This was significant with *Dex* at 1-10 μM ($p < 0.005$) and with *Hdc* at 0.1-10 μM ($p < 0.05$). *Flu* (1-30 nM) inhibited NO production and iNOS expression only partially (~50%) and the effects were significant at 1-30 nM ($p<0.05$). *Mfp* at 10 μM was able to reverse the inhibitions caused by *Dex* (10μM), *Hdc* (10μM) and *Flu* (3 nM). In contrast, *Eprn* had no significant effect on

the inhibition of NO production and iNOS expression caused by any of the compounds. C16 reduced NO and iNOS in a concentration dependent manner which was significant between 0.1-1μM ($p < 0.05$).

Conclusions: The partial inhibition of iNOS and NO caused by *Flu* suggest that the action of *Dex* and *Hdc* are not restricted solely to glucocorticoid receptors and other receptors and/or pathways may also be affected by these two compounds in causing the complete inhibition of iNOS in vascular smooth muscle cells. The mineralocorticoid receptor does not appear to be involved, while PKR may play a critical role in regulating the expression and function of iNOS in vascular smooth muscle cells.

References:

1. Cooke JP & Dzau VJ (1997). *Annual Review of Medicine* **48**: 489-509.
2. Thakur S & Baydoun AR (2012). *Amino Acids* **43**: 667-676.