

## **How Does Stress Increase Smoking Behaviour?**

### **Effect of Glucocorticoids on Nicotine Metabolism**

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#### **Background, purpose and hypothesis:**

Nicotine is a toxin found in tobacco products that is the main determinant of tobacco addiction. When tobacco is smoked or chewed, nicotine is absorbed into the bloodstream and is carried to the liver. Through a process called metabolism, liver enzymes called cytochrome P450s (CYPs) change nicotine into a water-soluble form that can then be eliminated in the urine. Specifically, these enzymes are CYP2A6 in humans and CYP2A5 in mice. Since these enzymes have very similar DNA sequences, protein structures and functional properties, nicotine metabolism can be studied in mice. High CYP2A5 and CYP2A6 activity increases nicotine metabolism and lowers nicotine levels in the blood. Since smokers consume tobacco to maintain blood nicotine levels, a higher rate of metabolism will increase the urge to smoke.

While smoking behavior increases with stress, the effect of stress-released glucocorticoid hormones on nicotine metabolism has not been studied. The purpose of this project is to determine whether glucocorticoids increase nicotine metabolism in liver cells called hepatocytes. I hypothesize that glucocorticoids such as dexamethasone (a man-made drug) and cortisol (a natural hormone) increase the amount of CYP2A5 and the rate of nicotine metabolism in mouse hepatocytes.

**Procedure :**

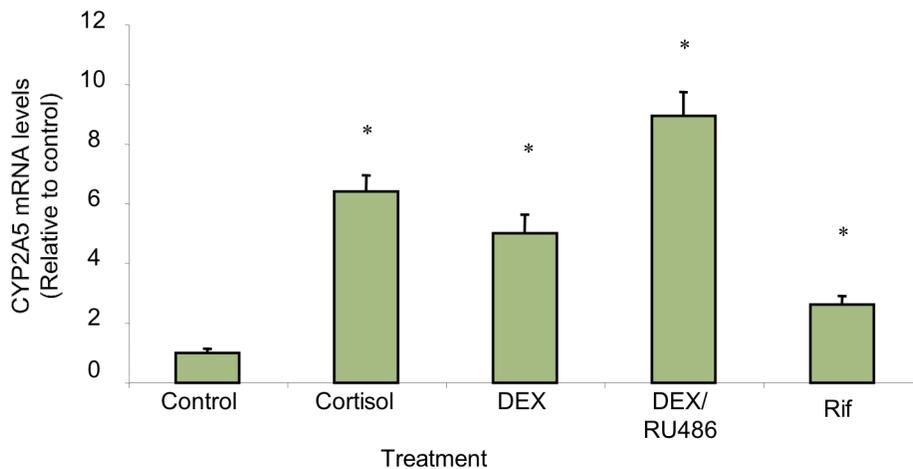
The effect of glucocorticoids on the amount of CYP2A5 in hepatocytes was measured at the mRNA level by Real-time PCR (Polymerase Chain Reaction) and by measuring nicotine metabolism in vitro by high performance liquid chromatography (HPLC) analysis of metabolites. Mouse hepatocytes were treated in 6 well plates (2.5 million cells/well) with 1  $\mu$ M of dexamethasone (DEX), 10  $\mu$ M of cortisol, 1  $\mu$ M of the glucocorticoid antagonist RU486, 10  $\mu$ M of the pregnane X receptor (PXR) agonist rifampicin (Rif) and control cells with 0.01% of dimethylsulfoxide (DMSO) for 24 hours.

To measure mRNA levels, cells were then harvested into a chemical called TRIzol to maintain the integrity of the mRNA. The total amount of RNA was then quantified using a Nanodrop spectrophotometer. To determine the amount of CYP2A5 mRNA, it was first reverse transcribed into cDNA using Murine Moloney Leukemia Virus reverse transcriptase and random primers. CYP2A5 cDNA was then amplified by RT-PCR using a Roche Lightcycler and fluorescent SYBR green dye and quantified by measuring fluorescence.

To measure CYP2A5 enzyme activity, cells were harvested, homogenized in a 1.5 mM Tris-Cl buffer and incubated with 3  $\mu$ M of nicotine, 1 mg/mL of cytosol (as a source of aldehyde oxidase) and 1 mM NADPH for 30 min at 37°C. Reactions were stopped, caffeine (250 ng) was added as an internal standard, and nicotine metabolites were extracted with dichloromethane on ISOLUTE HM-N columns. Metabolites were recovered under a stream of nitrogen gas and analyzed by HPLC with UV detection using a C18 reverse-phase column and a mobile phase of acetonitrile/potassium phosphate buffer.

## Results:

**CYP2A5 mRNA:** The first objective was to determine whether glucocorticoids increase the amount of CYP2A5 in mouse hepatocytes. DEX and cortisol both increased CYP2A5 mRNA levels by 5-6 fold compared to controls. To understand how this increase occurs and whether the GR was involved, GR was blocked with the antagonist RU486 before treating the hepatocytes with DEX. Co-treatment of hepatocytes with RU486 and DEX further increased CYP2A5 mRNA to levels over 8 fold greater than



**Figure 1:** DEX and cortisol increase CYP2A5 mRNA levels by 5-6 fold

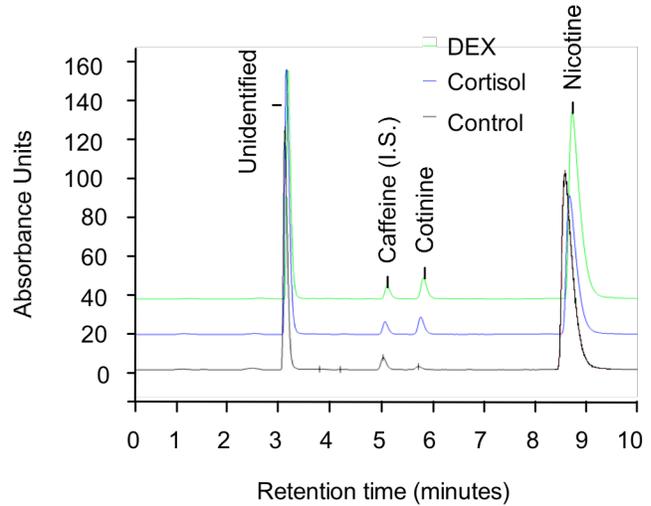
controls (Fig. 1). Because RU486 also activates PXR, increases of CYP2A5 may involve this nuclear receptor. The fact that the PXR agonist rifampicin (Rif) increased CYP2A5 mRNA by 2.6 fold support that suspicion.

**Nicotine metabolism:** To determine whether increases in CYP2A5 mRNA levels by glucocorticoids influence nicotine metabolism, nicotine metabolites were analyzed by mouse hepatocytes treated with DEX and cortisol (Fig 2). Peak areas of the major

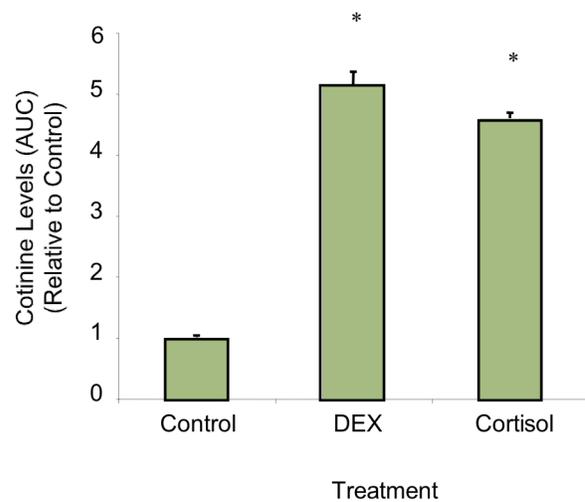
nicotine metabolite cotinine were increased by approximately 5 fold in DEX and cortisol-treated hepatocytes compared to controls (Fig 3).

**Conclusions:**

This is the first time that DEX and cortisol, at concentrations equivalent to levels that occur during stress, have been shown to increase the expression of nicotine-metabolizing CYP2A5 in mouse hepatocytes. This increase does not appear to occur through the GR but may involve the nuclear receptor PXR because the PXR activator rifampicin also increases CYP2A5 expression. The increase in CYP2A5 mRNA levels by DEX and cortisol also greatly increased the amount of nicotine metabolized by mouse hepatocytes. These findings suggest that glucocorticoids released during stress may increase the urge to smoke by speeding up the metabolism and removal of nicotine from the blood.



**Figure 2:** Cotinine metabolite peaks were increased by DEX and cortisol



**Figure 3:** DEX and cortisol increased the cotinine metabolite levels by approximately 5 fold

### **Acknowledgements:**

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