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Abstract # 1549

INCREASED HYPOTHALAMIC DOPAMINERGIC NEURON TYROSINE HYDROXYLASE EXPRESSION IN LEAN WISTAR RATS.

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Introduction

Calorific restriction in Wistar rats has been reported to show a decrease in mammary and pituitary tumours and an increase in uterine tumours associated with delayed hypothalamic dopaminergic neuronal senescence. This study was designed to assess the effects of compound induced reduced body weight gain on hypothalamic dopaminergic neurons using FFPE tissue obtained from 100 female Wistar rats (50 controls; 50 with >30% body weight gain reduction) from a 2 year bioassay. A combination of immunostaining (IHC) and RNAscope™ *in situ* hybridisation (ISH) was used to examine expression of tyrosine hydroxylase (TH), a rate limiting enzyme for dopamine synthesis.

Female Rat Endocrinology: Dietary (Caloric) Restricted Rats

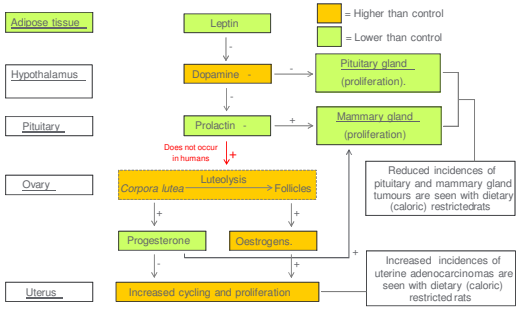


Fig 1: Rationale behind the study and hypothesis

Methods

IHC

Rat brain (hypothalamus) FFPE tissue samples were immunostained according to the following methods: Following HIER in NCL pH6 retrieval buffer, sections were stained using a vision biosystems Bond x staining robot using a refine 120,15 protocol. Sections were dehydrated with xylene, coverslipped with pertex and imaged using a Olympus Provis research microscope. The primary anti-tyrosine hydroxylase antibody (Abcam, Ab211) was used at a 1:20,000 dilution. Montages were prepared in Adobe photoshop.To examine the relationship between TH protein vs RNA staining in more detail dual fluorescence IHC/ISH was performed.

RNAscope™ *in situ* hybridisation staining

RNAscope™ *in situ* hybridisation (ISH) was performed on rat brain FFPE sections as described in the Advanced Cellular Diagnostics method entitled RNAscope® 2.0 FFPE Assay User Manual Brown. The number of DAB positive areas per field and the total positive DAB stained area (μm^2) per field were calculated.

Microarray and Pathways Analysis

Laser dissection of FFPE tissue samples was carried out using the Zeiss PALM Microbeam. RNA was extracted using the Qiagen *mi*RNeasy FFPE Kit. mRNA and *mi*RNA arrays were carried out. Signature lists of differentially expressed genes were generated using R and GeneSpring GX Software. Unbiased and biased pathways analysis was carried out using Ingenuity pathways analysis.

Results

Microscopic examination revealed TH protein (IHC) expression was apparent in both the Arcuate Nucleus (Tubular Infundibular DopA neurons – TIDA) and the median eminence (ME), whilst the TH messenger RNA (ISH) staining was largely restricted to the TIDA neurons (Fig 2). There was an apparent increase in TH staining (IHC and ISH) in those animals from the group which showed reduced body weight gain, compared to the control animals (Fig 2), which was confirmed by image analysis (Table 1).

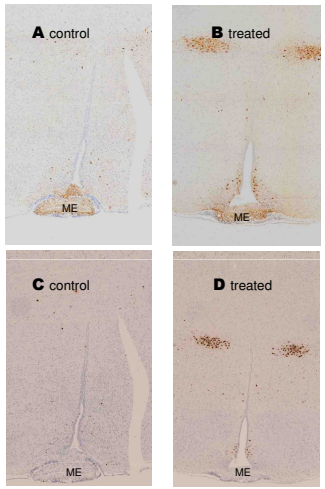


Fig 2: Hypothalamus TH IHC/ISH : (A) TH IHC control (B) TH IHC treated (C) TH ISH control (D) TH ISH treated.

Table 1: ISH/IHC Image analysis data derived from analysis of TIDA ROIs. Results are mean (DAB+threshold/ROI area) ± SD (n=6), statistically significantly different from control ,
*** p<0.05, **P<0.005, students T1 tailed type 2 test. (ROI = region of interest)**

RNAscope ISH (RNA)				IHC (protein)			
Animal	DAB+ area [uM²]	Mean DAB+ ROI	SD	Animal	DAB+ area [uM²]	Mean DAB+ ROI	SD
control				control			
262	645.9			262	494.7		
269	783.2			269	678.1		
292	162.4			292	5506.4		
293	2521.2			293	3680.2		
296	267.7	1018.6	949	296	2220.2	2688.7	2118.4
306	605.9			306	2312.7		
263	492.4			263	1264.3		
276	157			276	189.9		
288	2335.5			288	4701		
291	2215.2			291	5839.2		
treated				treated			
453	929.6			453	7707.6		
454	3719.7			454	2132.4		
471	5717.6			471	7061.7		
472	3838.6			472	3061.3		
481	5679.2			481	3207.6		
482	3329	3780.7**	2374.2	482	5380.6	4884.9*	2894.8
457	2232.7			457	1710.3		
462	2322.9			462	2505.2		
465	2703			465	4379		
480	1363.3			480	3270.8		
491	3855.1			491	6718.6		
494	9677.8			494	11484		

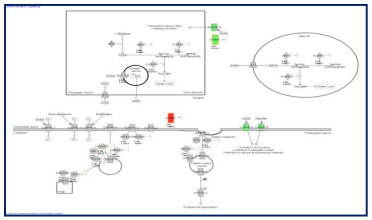
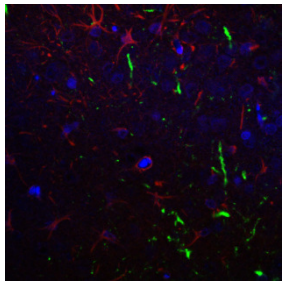


Fig 3: IPA classical pathway GABA receptor signalling showing GABA B receptor down-regulated and GABA receptor theta up-regulated by the treatment.

- Laser dissection targeted (LDT) microarray and pathways analysis showed a CAR-agonist 'signature' in liver.
- Up-regulation of TH and down-regulation of GABA B receptors in hypothalamus
- Down-regulation of prolactin in pituitary and up-regulation of ERα in uterus.

Fig 4: Dual IHC staining of leptin receptor (red) and TH (green) in rat hypothalamus showing that leptin receptor expression on gabergic neurons does not co-localise with TH expression.



Conclusions

•Chronic reduction in bodyweight gain causes delayed senescence of dopaminergic neurons as reflected by increased TH expression (relative to control), in hypothalamic nuclei due to reduced GABA B receptor signalling mediated by a reduction in leptin signalling to gabergic neurons (Vong *et al.* 2011) .

•This in turn causes inhibition of prolactin secretion by pituitary and consequent up-regulation of estrogen production in the ovaries resulting in increased cycling and proliferation of the uterine epithelium.

Ref: Vong *et al.* 2011 *Neuron* **71**, 142–154.

This study was funded by Syngenta