PRODYNORPHIN GENE REGULATION BY EPIGENETIC MECHANISMS FOLLOWING ETHANOL AND ACETALDEHYDE EXPOSURE IN SH-SY5Y CELLS

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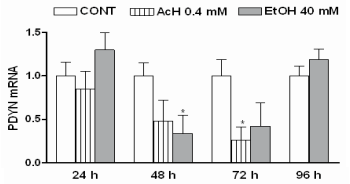
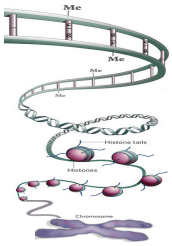
INTRODUCTION and AIMs

Ethanol (EtOH) alters neural activity through interaction with multiple neurotransmitters and the endogenous opioid system seems to play a key role in its action. We have recently investigated the transcriptional regulation of opioid system genes in response to exposure to EtOH up to 96 h and acetaldehyde (AcH) up to 72 h in human neuroblastoma SH-SY5Y cells (**D´Addario et al, 2008; see fig**).

Among the opioid precursor genes down-regulated, we further investigated the **prodynorphin (PDYN)** gene to better clarify the importance of AcH for EtOH neurotoxicity **studying the potential epigenetic mechanisms** of histone 3 trimethylation of lysine-4 (H3K4me3), lysine-9 (H3K9me3) and lysine-27 (H3K27me3) and DNA methylation.



METHODS

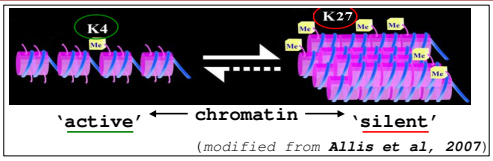


(*from* **Qiu, 2006**)

Study of the epigenetic code:

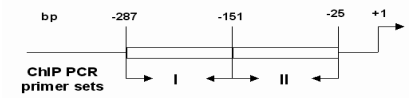
✓ **DNA methylation** (gene REPRESSION)
- by LUMA (Global DNA methylation)
- by bisulfite-pyrosequencing
(PDYN promoter methylation)

✓ **Histone modifications**
- by Chromatin Immunoprecipitation followed by Real-Time PCR [position and size of primer sets used (**I** and **II**) on PDYN promoter are shown]



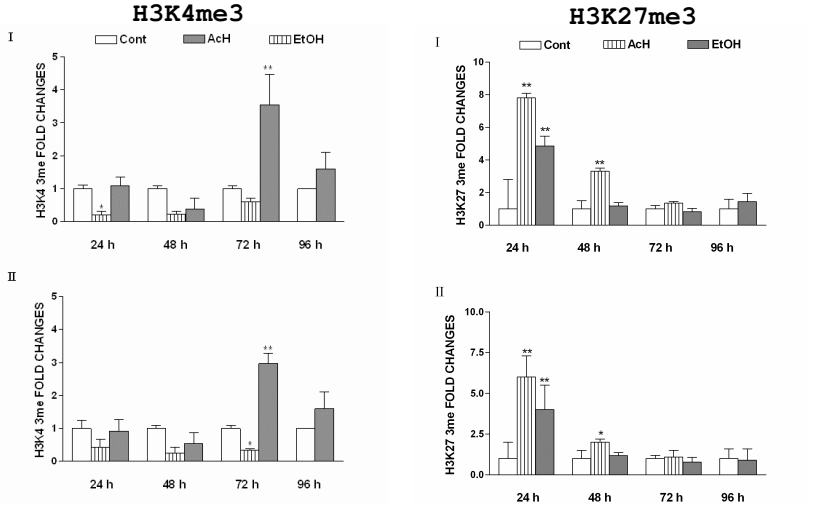
(*modified from* **Allis et al, 2007**)

PDYN proximal promoter region



RESULTS

Histone modifications



DNA Methylation

CpGs in PDYN promoter

	24 h				48 h				72 h			
	% Methylation at CpG sites				% Methylation at CpG sites				% Methylation at CpG sites			
	CpG 1	CpG 2	CpG 3	mean	CpG 1	CpG 2	CpG 3	mean	CpG 1	CpG 2	CpG 3	mean
CONT	26,5	42,7	26,7	**32,0**	30,7	45,0	25,6	**33,8**	22,3	42,3	23,8	**29,5**
AcH	26,3	40,8	23,9	**30,3**	29,5	47,1	26,7	**34,5**	25,0	39,0	22,6	**28,9**
EtOH	24,7	44,5	27,2	**32,1**	31,1	46,4	27,2	**34,9**	23,6	43,6	26,7	**31,3**

general

	24 h		48 h		72 h	
	HpaII/EcoRI	HpaII/MspI	HpaII/EcoRI	HpaII/MspI	HpaII/EcoRI	HpaII/MspI
CONT	0,53 ± 0,06	0,30 ± 0,013	0,60 ± 0,06	0,30 ± 0,006	0,52 ± 0,02	0,33 ± 0,015
AcH	0,53 ± 0,06	0,28 ± 0,017	0,59 ± 0,04	0,34 ± 0,008	0,46 ± 0,04	0,32 ± 0,014
EtOH	0,53 ± 0,09	0,31 ± 0,019	0,57 ± 0,04	0,32 ± 0,008	0,51 ± 0,04	0,33 ± 0,006

Changes in **histone modifications** (H3K4me3 and H3K27me3) in the amplicons I and II in the human PDYN proximal promoter region and **DNA methylation** (global and of 3 CpGs on PDYN promoter) in SH-SY5Y cells following exposure to EtOH (40 mM) or AcH (0.4 mM) at different time points [** P < 0.01, * P < 0.05 vs control; ANOVA and Dunnett's test]

CONCLUSIONS

✓ we demonstrated a synchronous colocalization of H3K27me3 and H3K4me3 in the PDYN promoter possibly keeping the PDYN gene in a poised state for later activation

✓ reactivation of PDYN gene by the same agents upon prolonged exposure, may be related to preferential methylation of H3K4 and an unmethylated H3K27

✓ the effect showed selectivity, since neither lysine-9 (H3K9) nor DNA methylation were altered

✓ our data provide further evidence of the important role of AcH in mediating the EtOH-induced effects.

REFERENCES

D'Addario C, Ming Y, Ogren SO, Terenius L (2008) FASEB J 22, 662-670.
Allis CD, Jenuwein T, Reinberg D (2007) Epigenetics Cold Spring Harbor Laboratory Press.
Qiu J (2006) Nature 441, 143-145.

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No potential conflict of interest