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ORIGINAL ARTICLES

Differential regulation of proopiomelanocortin (POMC) mRNA expression in hypothalamus and anterior pituitary following repeated cyanamide with ethanol administration

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Background/Aim. *We have investigated proopiomelanocortin (POMC) mRNA expression in the arcuate nucleus of the hypothalamus (ARC) and the anterior lobe of the pituitary (AL) following repeated cyanamide-ethanol reaction (CER).* ***Methods.*** *Adult male Sprague -Dawley rats (250–290 gr) were housed in a temperature and humidity controlled environment with free access to food and water. Four experimental groups were used as follows: saline (as control), cyanamide alone, ethanol alone and ethanol with cyanamide. The animals recived daily intraperitoneal injections (i.p.) of cyanamide (10mg/kg, 60 min before ethanol dosing) with or without ethanol (1g/kg) for 5 consecutive days, and were sacrificed 60 min after the last dosing of ethanol. The results were presented as the mean ± SEM for each group. All groups within each deta set were compared by one-way ANOVA followed by Fisher PLSD test for multiple comparisons. A value of $p<0.05$ was considered significant.* ***Results.*** *The POMC mRNA levels in ARC were significantly decreased with cyanamide compared to the control and ethanol alone ($p<0.05$ and $p<0.05$ respectively), but increased in AL following repeated CER.* ***Conclusion.*** *We speculate that this differential regulation of POMC mRNA expression may be partially involved in the preventive effects on alcohol intake in response to CER.*

K e y w o r d s : **RNA, messenger; hypothalamus; pituitary gland; cyanamide; ethanol.**

Introduction

Alcoholism is a serious problem in many developed countries. It is generally accepted that the endogenous opioid system, including β-endorphin (β-EP) plays a key role in alcohol addiction (1, 2). It has been reported that alcohol consumption is reduced following treatment with opioid receptor antagonists (3, 4). These data suggest a strong involvement of β-EP in ethanol addiction. β-EP is the product of posttranslational processing of proopiomelanocortin (POMC) (5, 6), and the major site of synthesis in the brain is the arcuate nucleus (ARC) (5). It is believed that β-EP in brain may be involved in reward mechanisms (2). The anterior lobe (AL) and neurointermediate lobe of the pituitary also represent a major source of POMC production (5). POMC mRNA levels in the AL are increased in response to a wide variety of stressful stimuli (7).

Cyanamide, a potent inhibitor of aldehyde dehydrogenase, is widely used as an anti-alcoholic drug (8, 9). It produces severe uncomfortable symptoms such as flushing, tachycardia and hypotension, when it is used with ethanol, due to the accumulation of acetaldehyde in blood (10). It is believed that these unpleasant symptoms (cyanamide-ethanol reaction –CER) provide a potent aversive feeling to alcohol. A possible neuronal mechanism underlying CER remains to be elucidated. In this study, we have investi-

gated POMC mRNA expression in ARC and AL following repeated CER. We have also investigated corticotrophin releasing factor (CRF) mRNA expression in the paraventicular nucleus (PVN). This study provides the first report of the possible contribution of neuronal mechanisms underlying CER.

Methods

Adult male Sprague-Dawley rats (250–290gr) were housed in a temperature and humidity controlled environment with free access to food and water. All experimental procedures were performed on a project licence granted under the terms of the Animal (Scientific Procedures) Act 1986. Four experimental groups were used as follows, saline (as control), cyanamide alone, ethanol alone and ethanol with cyanamide. The animals received daily intraperitoneal injections (i.p.) of cyanamide (10mg/kg, 60min before ethanol dosing) with or without ethanol (1g/kg) for 5 consecutive days, and were sacrificed 60 min after the last dosing of ethanol. This dose of cyanamide or ethanol has previously been shown to be insufficient to stimulate hypothalamic CRF or POMC mRNA expression when administrated alone (11, 12). We chose this dose for optimal condition to observe CER. The brains and pituitary glands were rapidly removed from the skull, snap frozen on dry-ice and kept at -80° C until sectioning. Coronal sections, 12µm-thickness, containing the ARC and PVN in brain and pituitaries were cut and thaw-mounted on gelatin-coated slides and stored at -80° C before hybridization.

In situ hybridization histochemistry was performed as described previously (13). The probes were oligonucleotides (14, 15), and were labeled at the 3' end with [^{35}S]-dATP (1 000µCi/mmol, NEN, Boston, MA, USA). The specific activities of the probes were 5.48×10^{18} and 1.18×10^{19} dpm/mol for POMC and CRF, respectively. Approximately 100 000cpm probe were applied to each slide, and all the sections for each hybridization were processed at the same time. The sections were apposed to Hyperfilm MP autoradiography film (Amersham, UK). The autoradiographic images were measured using a computer-assisted image analysis system (Image 1.22) run on an Apple Macintosh, as described previously (13). The results were presented as the mean percentage change from eontrol, assigned an arbitrary value of 100, as the mean±SEM for each group. All groups within each data set were compared by one-way ANOVA followed by Fisher PLSD test for multiple comparisons. A value of $p<0.05$ was considered significant.

Results

POMC mRNA in the ARC was unaffected by either cyanamide or ethanol treatment alone. However, POMC mRNA was significantly decreased with combined treatment of ethanol with cyanamide compared to the control and ethanol alone $p<0.05$ and $p<0.05$, respectively (Figure 1a). In contrast, the expression of POMC mRNA was significantly increased in the AP in the group receiving ethanol with cyanamide, $p<0.05$ and $p<0.01$ compared to the control and ethanol alone, respectively (Figure 1b). CRF mRNA in the PVN was also significantly increased following combined treatment with ethanol and cyanamide compared to control and ethanol alone $p<0.001$ and $p<0.01$ respectively (Figure 1c).

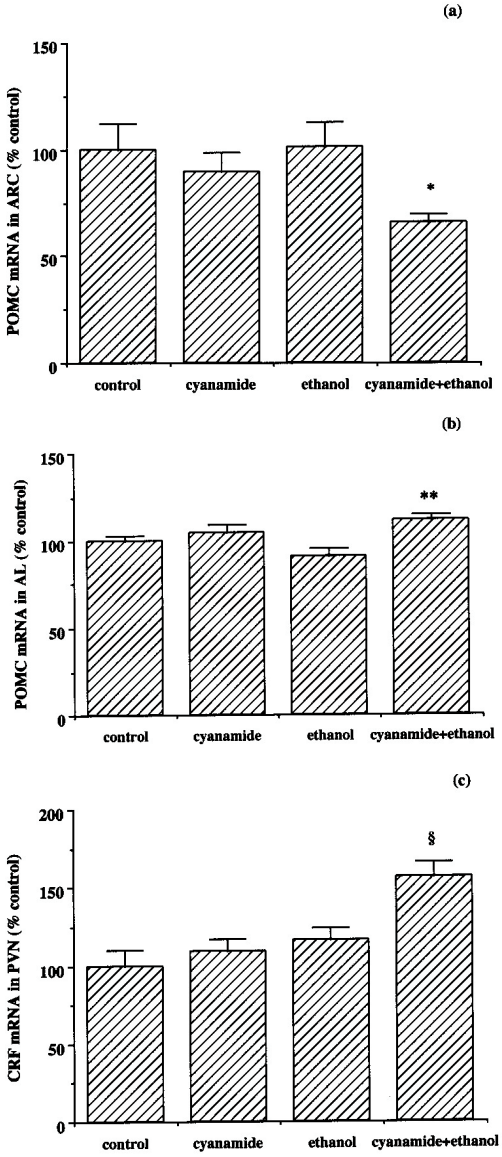

Fig. 1 – (a) proopiomelanocortin (POMC) mRNA levels in the arcuate nucleus (ARC), (b) POMC mRNA levels in anterior lobe of the pituitary (AL), (c) corticotrophin releasing factor (CRF) mRNA levels in the paraventricular nucleus (PVN) of rats following repeated administration of saline (control), cyanamide, ethanol and ethanol with cyanamide (cyanamide+ethanol). These are expressed as the percentage change from the eontrol group. Values are presented as meam±S.E.M. for n=7–8 rats/group.

*$p < 0.05$ compared with eontrol and ethanol.
**$p < 0.05$ compared with eontrol and p<0.01 compared with ethanol.
§$p < 0.001$ compared with eontrol and p<0.01 compared with ethanol.
(One-way ANOVA followed by Fisher PLSD test)

Discussion

The axons of β-EP expressing neurons in ARC project to many cerebral areas including limbic structures such as the ventral tegmental area and the nucleus accumbens (16). Cell bodies of the mesolimbic dopaminergic system originate from the ventral tegmental area and project to the nucleus accumbens and have been implicated in drug reinforcement (17). The activity of these dopaminergic neurons could also be regulated by β-EP neurons (2, 18). As the content of mRNA in a tissue is usually associated with the rate of biosynthesis and release of its specific peptide (19), the changes of POMC mRNA expression may be associated with changes in β-EP release. In this study, we have demonstrated a decrease in POMC mRNA in ARC by CER. This may represent a decrease in mesolimbic dopaminergic activity by CER, and has a possibility to be associated with the prevention of alcohol intake by CER.

Our study also resulted in significant increases in both CRF mRNA in the PVN and POMC mRNA in the AP by CER. The increase of POMC mRNA in AL appears to be driven by an increase in CRF neuronal activity (20). Therefore, these data suggest the activation of the HPA axis at a central level by CER. Our observation suggest the CER act as not only a potent stressor, but may also regulate mesolimbic dopamine neurons via ARC β-EP neurons.

In conclusion, the present results indicate a differential effect of CER on POMC mRNA expression with a significant decrease in ARC POMC expression and a significant increase in the AL. We speculate that this differential regulation may be partially involved in the preventive effects on alcohol intake in response to CER. This could form an interesting target for the study of treatment of alcohol addiction. The detailed mechanisms underlying this difference are not clear and additional studies will be required to clari&y the involvement of β-EP.

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R E F E R E N C E S

1. *Gianoulakis C*. The effect of ethanol on the biosynthesis and regulation of opioid peptides. Experientia 1989; 45(5): 428–35.
2. *Herz A*. Endogenous opioid systems and alcohol addiction. Psychopharmacology (Berl) 1997; 129(2): 99–111.
3. *Altshuler HL, Phillips PE, Feinhandler DA*. Alteration of ethanol self-administration by naltrexone. Life Sci 1980; 26(9): 679–88.
4. *Krishnan-Sarin S, Wand GS, Li XW, Portoghese PS, Froehlich JC*. Effect of mu opioid receptor blockade on alcohol intake in rats bred for high alcohol drinking. Pharmacol Biochem Behav 1998; 59(3): 627–35.
5. *Pelletier G*. Regulation of proopiomelanocortin gene expression in rat brain and pituitary as studied by in situ hybridization. Ann N Y Acad Sci 1993; 680: 246–59.
6. *Raffin-Sanson ML, de Keyzer Y, Bertagna X*. Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions. Eur J Endocrinol 2003; 149(2): 79–90.
7. *Harbuz MS, Lightman SL*. Responses of hypothalamic and pituitary mRNA to physical and psychological stress in the rat. J Endocrinol 1989; 122(3): 705–11.
8. *Sellers EM, Naranjo CA, Peachey JE*. Drug therapy: Drugs to decrease alcohol consumption. N Engl J Med 1981; 305(21): 1255–62.
9. *Peachey JE, Naranjo CA*. The role of drugs in the treatment of alcoholism. Drugs 1984; 27(2): 171–82.
10. *Johnsen J, Stowell A, Morland J*. Clinical responses in relation to blood acetaldehyde levels. Pharmacol Toxicol 1992; 70(1): 41–5.
11. *Kinoshita H, Jessop DS, Finn DP, Coventry TL, Roberts DJ, Ameno K,* et al. Acute ethanol decreases NPY mRNA but not POMC mRNA in the arcuate nucleus. Neuroreport 2000; 11(16): 3517–9.
12. *Kinoshita H, Jessop DS, Finn DP, Coventry TL, Roberts DJ, Ameno K,* et al. Acetaldehyde, a metabolite of ethanol, activates the hypothalamic-pituitary-adrenal axis in the rat. Alcohol Alcohol 2001; 36(1): 59–64.
13. *Harbuz MS, Jessop DS, Lightman SL, Chowdrey HS*. The effects of restraint or hypertonic saline stress on corticotrophin-releasing factor, arginine vasopressin, and proenkephalin A mRNAs in the CFY, Sprague-Dawley and Wistar strains of rat. Brain Res 1994; 667(1): 6–12.
14. *Young WS 3rd, Mezey E, Siegel RE*. Quantitative in situ hybridization histochemistry reveals increased levels of corticotropin-releasing factor mRNA after adrenalectomy in rats. Neurosci Lett 1986; 70(2): 198–203.
15. *Levy A, Lightman SL*. Quantitative in-situ hybridization histochemistry in the rat pituitary gland: effect of bromocriptine on prolactin and pro-opiomelanocortin gene expression. J Endocrinol 1988; 118(2): 205–10.
16. *Finley JC, Lindstrom P, Petrusz P*. Immunocytochemical localization of beta-endorphin-containing neurons in the rat brain. Neuroendocrinology 1981; 33(1): 28–42.
17. *Koob GF*. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci 1992; 13(5): 177–84.
18. *de Waele JP, Kiianmaa K, Gianoulakis C*. Distribution of the mu and delta opioid binding sites in the brain of the

alcohol-preferring AA and alcohol-avoiding ANA lines of rats. J Pharmacol Exp Ther 1995; 275(1): 518–27.

19. *Marinelli PW, Kiianmaa K, Gianoulakis C*. Opioid propeptide mRNA content and receptor density in the brains of AA and ANA rats. Life Sci 2000; 66(20): 1915–27.

20. *Affolter HU, Reisine T*. Corticotropin releasing factor increases proopiomelanocortin messenger RNA in mouse anterior pituitary tumor cells. J Biol Chem 1985; 260(29): : 15477–81.

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A p s t r a k t

Kinoshita K, Hishida S, Ameno K, Ijiri I, Harbuz MS. Vojnosanit Pregl 2005; 62(12): : 875–878.

DIFERENCIJALNA REGULACIJA EKSPRESIJE PROOPIOMELANOKORTINA (POMC) iRNK U HIPOTALAMUSU I PREDNJEM REŽNJU POSLE PONOVLJENOG DAVANJA CIANAMIDA SA ETANOLOM

Uvod/Cilj. Ispitati ekspresiju proopiomelanokortina (POMC) iRNK u nukleus arkuatusu hipotalamusa (ARC) i u prednjem režnju (AL) hipofize posle ponovljene reakcije cijanamid-etanol (CER). **Metode.** Odrasli mužjaci Sprague-Dawley pacova su smešteni u okruženje sa kontrolisanom temperaturom i vlažnošću i sa slobodnim pristupom hrani i vodi. Četiri eksperimentne grupe korišćene su na sledeći način: slani rastvor (kontrolna), samo cijanamid, samo etanol i etanol sa cijanamidom. Životinje su dobijale dnevno intraperitonealne injekcije (i.p.) cijanamida (10mg/kg, 60 min pre doziranja etanola) sa ili bez etanola (1 g/kg) tokom 5 uzastopnih dana, pa su žrtvovane 60 min posle zadnjeg doziranja etanola. Rezultati su izraženi kao srednja vrednost i SD za svaku grupu. Sve grupe su unutar svakog kompleta podataka upoređene pomoću jednosmernog ANOVA testa, pa zatim Fisher PLSD testa za višestruko upoređivanje. Vrednost od $p < 0,05$ je smatrana značajnom. **Rezultati.** Nivoi POMC iRNK u ARC su značajno opali pomoću cijanamida u poređenju sa kontrolnom grupom i samo etanol grupom ($p < 0,05$ i $p < 0,05$ očekivano), dok su u AL, međutim, porasli posle ponovljene CER. **Zaključak.** Smatramo da bi se ova mogućnost različitog uticaja na ekspresiju POMC iRNK mogla delimično iskoristiti za prevenciju alkoholizma.

K lj u č n e r e č i : **RNK, informativna; hipotalamus; hipofiza; cijanati; alkohol, etil.**

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