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Research Report

Ethanol activates cAMP response element-mediated gene expression in select regions of the mouse brain

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Abbreviations:
Calpha, PKA catalytic subunit
pCREB, phosphorylated cAMP response element
CRE, cAMP response element
beta-gal, beta-galactosidase
NAc, nucleus accumbens
PFC, prefrontal cortex
BLA, basolateral amygdala
HC, hippocampus
SNc, substantia nigra compacta
VTA, ventral tegmental area

ABSTRACT

The specific brain regions that contribute to behavioral changes produced by ethanol are not clearly understood. We know that cAMP-PKA signaling has been strongly implicated in the CNS effects of ethanol. Ethanol promotes activation and translocation of the PKA catalytic subunit (Calpha) into the nucleus in cell lines and primary neuronal cultures. PKA Calpha translocation to the nucleus is followed by cAMP Response Element protein phosphorylation (pCREB) and cAMP Response Element (CRE)-mediated gene expression. Here, we use X-gal histochemistry to map CRE-mediated gene transcription in the brain of CRE-lacZ transgenic mice following ethanol injection. Results: 3 h after i.p. ethanol injection (3.2 g/kg, 16% wt/vol.), the number of X-gal positive cells was increased in the nucleus accumbens (202 ± 63 cells/field compared to 71 ± 47 cells/field in saline injected controls, $P < 0.05$ by paired t-test, $n = 10$). Similar increases were found in other mesolimbic areas and brain regions associated with rewarding and addictive responses. These include: prefrontal cortex, lateral and medial septum, basolateral amygdala, paraventricular and anterior hypothalamus, centromedial thalamus, CA1 region of hippocampus and dentate gyrus, substantia nigra pars compacta, ventral tegmental area, geniculate nucleus and the superior colliculus. Conclusion: these results confirm and extend current concepts that ethanol stimulates cAMP-PKA signaling in brain regions involved in CNS responses to ethanol.

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Activation of cAMP/PKA signaling appears to be a major response to ethanol in NG108-15 cells and primary neurons in culture (Diamond and Gordon, 1997; Mailliard and Diamond, 2004; Yao et al., 2002). In these cell culture systems, ethanol inhibits adenosine uptake and increases extracellular adenosine levels, causing activation of adenosine A2A receptors (A2A) and Golf-mediated increases in cAMP production. cAMP-dependent PKA is a heterodimer consisting of two regulatory (R) and two catalytic subunits (Calpha). cAMP promotes PKA activity by binding to R subunits, thereby releasing active

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Calpha subunits. Localization of PKA and other kinases in the cell determines their substrate specificity (Dell'Acqua and Scott, 1997; Lester and Scott, 1997; Taylor et al., 2005). We have shown that ethanol promotes A2A-dependent PKA Calpha translocation to the nucleus in cultured neural cells (Dohrman et al., 1996, 2002; Yao et al., 2002). Ethanol-induced PKA Calpha in the nucleus remains functionally active as long as ethanol is present. Persistent activation and localization of Calpha in the nucleus appears to be due, in part, to ethanol inhibition of Calpha reassociation with R subunits as well as ethanol interference with PKA binding to PKI, a PKA inhibitor that exports PKA out of the nucleus (Constantinescu et al., 2002).

Ethanol-induced PKA Calpha translocation into the nucleus appears to have pathophysiologic significance. PKA catalyzes CREB phosphorylation (pCREB) on Ser-133, initiating transcription of CRE-containing genes. Previously, we have reported that ethanol increases pCREB within minutes in NG108-15 cells (Constantinescu et al., 1999). Using a luciferase reporter construct under CRE control, we have also shown that ethanol promotes PKA-dependent CRE-mediated gene expression hours later (Asher et al., 2002, Yao et al., 2002).

PKA, pCREB and cAMP-dependent CRE-mediated transcription have been implicated in alcoholism (Thiele et al., 2000; Pandey et al., 2001, 2003, 2005; Wand et al., 2001; Zhang and Pandey, 2003; Yang et al., 2003; Misra and Pandey, 2005;Ferraro et al., 2006). Alcohol preferring rats, which exhibit increased ethanol consumption, express increased pCREB activity selectively in the central amygdala after acute ethanol exposure (Pandey et al., 2005). Many studies deal with the effects of ethanol on pCREB (Yang et al., 1998; Pandey et al., 2001, Li et al., 2003; Yang et al., 2003). However, phosphorylation of CREB alone is not sufficient to induce CRE-mediated gene transcription (Sugiura et al., 2004). Nevertheless, Miles and colleagues have reported that exposure to ethanol induces the expression of a variety of genes, many dependent on cAMP (Thibault et al., 2000), suggesting that ethanol-induced changes in cAMP/PKA signaling characterizes, in part, CNS responses to ethanol.

Several studies suggest that single exposure to ethanol appears to have persistent functional consequences. Thus, PKA-dependent changes in synaptic plasticity after a single injection of ethanol predispose mice to consume more ethanol (Spanagel and Weiss, 1999; Melis et al., 2002; Camarini and Hodge, 2004). Importantly, the PKA requirement for increased drinking appears to be associated with ethanol induced changes in GABA receptor activity. Thus, a single dose of ethanol potentiates PKA-dependent GABAergic synaptic function for more than 7 days (Melis et al., 2002).

However, the regional anatomy of ethanol-induced CRE-mediated gene transcription is incompletely understood. CRE-mediated gene transcription has been used as a marker for changes in synaptic plasticity (Impey et al., 1998; Obrietan et al., 1999; Thome et al., 2000; Barth et al., 2000; Shaw-Lutchmen et al., 2003; Athos et al., 2002; Barrot et al., 2002; Cancedda et al., 2003). Therefore, we asked whether ethanol-induced CRE-mediated gene transcription could be used to map the functional neuroanatomy of CNS responses to ethanol.

To identify brain regions in which CRE-mediated transcription is activated during acute ethanol exposure, we used transgenic CRE-reporter mice, in which the reporter gene, lacZ (encoding beta-galactosidase (beta-gal)) is under the control of a CRE-consensus sequence. Low to moderate doses of ethanol activate the dopaminergic pathway of the brain, which is strongly linked to reward and addiction, while high doses of ethanol can produce anesthetic and toxic effects and suppress dopaminergic activity (Budygin et al., 2005). We used the mid-range dose of ethanol (3.2 g/kg) because it does not cause sedation, as measured by preservation of the righting reflex in ethanol treated LacZ mice.

Here we report that acute exposure to ethanol results in a significant increase in CRE-mediated gene transcription in specific regions of the brain. These areas include the mesolimbic system and brain regions involved in psychomotor responses.

1. Results

1.1. *Activation of CRE-mediated transcription in the nucleus accumbens (NAc) in response to ethanol*

CRE activation in CRE-lacZ transgenic mice induces lacZ expression, which codes for beta-gal. X-gal is a chromogenic substrate for beta-gal that turns blue upon hydrolysis. Hence, X-gal is a marker for CRE-mediated transcription in the transgenic CRE-lacZ mice. X-gal staining is localized to the nucleus. Three fields selected at random were imaged in the shell-core area of the NAc (Fig. 1A). Despite considerable variation between experiments (due to heterogeneous expression of lacZ in animals), X-gal staining was 2.8-fold higher in ethanol-treated mice (3.2 g/kg), as compared to saline injected mice (ethanol 202 ± 63 cells/field; control, 71 ± 47 cells/field, $P < 0.05$ by paired t-test, $n = 10$ in each group) (Fig. 1B–D). Half of the ethanol dose (1.6 g/kg) produced half the increase (1.5-fold) in X-gal staining, (ethanol, 345 ± 78 cells/field; control, 232 ± 62 cells/field, $P < 0.05$ by paired t-test, $n = 10$ in each group), suggesting a proportionality of dose-response. The difference in the baseline between the two experiments is attributed to the heterogeneous expression of lacZ in the CRE transgenic mice. The group of mice used in the second experiment (1.6 g/kg) showed higher baseline lacZ expression.

1.2. *Regional neuroanatomy of ethanol-induced CRE-mediated gene transcription*

To identify brain regions selectively activated by ethanol, X-gal staining was mapped in the brains of ethanol-treated mice and compared to saline-treated controls. Three adjacent fields were imaged for each brain region in each animal, and the number of X-gal positive cells in each field was counted. Table 1 lists the mean ± the standard error of the mean (SEM) for each of those regions. X-gal staining was generally higher in the ethanol treated mice than in controls. Importantly, a significant ethanol-induced increase in X-gal staining was observed in the motor cortex, prefrontal cortex (PFC), lateral and medial septum, the NAc, the basolateral amygdala (BLA), the paraventricular and anterior hypothalamus, the centromedial thalamus, the CA1 region of the hippocampus (HC) and dentate gyrus, the substantia nigra pars compacta (SNc), the ventral tegmental area (VTA), the geniculate nucleus and the superior colliculus. There was no significant increase in the