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Rapid communication

Ethanol rapidly induces steroidogenic acute regulatory protein expression and translocation in rat adrenal gland

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Abstract

Acute ethanol exposure increases GABAergic neuroactive steroids in plasma and brain by releasing these steroids or their precursors from the adrenal glands. The present study showed that ethanol administration rapidly increases the expression of steroidogenic acute regulatory protein (StAR) in the cytosolic and mitochondrial fractions of adrenal glands. The increased StAR protein expression paralleled increases in plasma pregnenolone, progesterone and corticosterone levels. The rapid synthesis and translocation of StAR protein in adrenals likely represent the mechanism of ethanol-induced increases in neuroactive steroids.

Keywords: Ethanol; StAR (steroidogenic acute regulatory protein); Neuroactive steroid

Acute systemic ethanol administration elevates plasma and cerebral cortical GABAergic neuroactive steroids including progesterone, 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP, allopregnanolone) and 3α,21-dihydroxy-5α-pregnan-20-one (3α,5α-THDOC), contributing to behavioral effects of ethanol in rats (VanDoren et al., 2000; Khisti et al., 2002). Recently, we showed that adrenalectomy prevents the ethanol-induced elevation of cortical 3α,5α-THP while pretreatment with the immediate precursor of 3α,5α-THP, 5α-dihydroprogesterone, restores the ethanol-induced elevation of cortical 3α,5α-THP in adrenalectomized rats (Khisti et al., 2002, 2003). Hence, the ethanol-induced increase in plasma 3α,5α-THP is derived directly from the adrenal, while elevations in the brain require the release of steroid precursors from the adrenal gland. The precise mechanism of ethanol-induced elevation of neuroactive steroids in adrenal gland is not known.

Steroidogenic acute regulatory protein (StAR) is present in adrenal glands of rat, mouse and human, and plays an important role in steroidogenesis. StAR is synthesized as a 37-kDa cytosolic precursor protein and is processed into a mature 30-kDa protein during its transport into the mitochondria. Mitochondrial import of StAR protein rapidly increases (10–100 fold) steroidogenesis within minutes by regulating the transfer of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane (Bose et al., 2002). At the inner mitochondrial membrane, cholesterol side-chain cleavage enzyme $P450_{scc}$ converts cholesterol to pregnenolone (rate-limiting step). Recent studies have shown that StAR plays a critical role during stress and pregnancy. StAR protein is also present in detectable quantities in human and mouse brain (King et al., 2002). Since ethanol-induced increases in plasma and brain are adrenal-dependent, we studied the effect of acute ethanol administration on StAR protein expression in cytosolic and mitochondrial fractions of rat adrenal as well as plasma steroids including pregnenolone, progesterone and corticosterone.

Male Sprague–Dawley rats (250–300 g) handling habituated for 7 days with saline injections were administered ethanol (2 g/kg, 20% w/v in saline, i.p.) and sacrificed 60 min later. Adrenal glands and blood were rapidly collected and stored at −80 °C until use. Adrenal glands were homogenized in 0.32 M sucrose and the homogenate was subjected to high speed centrifugation to separate mitochondrial and cytosolic fractions (Gray and Whittaker, 1962). The fractions were subjected to western

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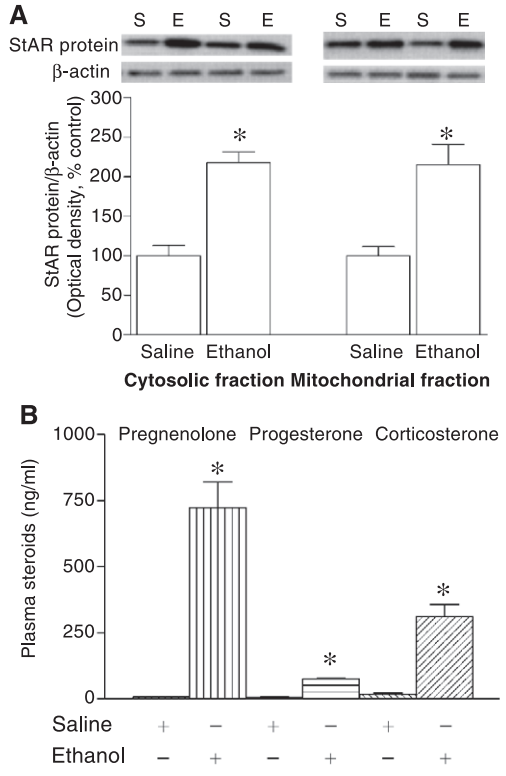

Fig. 1. Acute ethanol administration increases (A) adrenal StAR protein expression and (B) plasma neuroactive steroids. Male rats ($N=14$) were administered ethanol (2 g/kg, 20% w/v in saline) or saline, adrenal glands and blood were rapidly collected after 60 min. (A) Cytosolic and mitochondrial fractions were obtained by centrifugation (mitochondrial 17,000 × g; cytosolic 100,000 × g), subjected to gel electrophoresis using Novex Tris–Glycine gels (8–16%), transferred to polyvinylidene difluoride membranes and immunoblotted with rabbit StAR antibody (1:1000, Calbiochem). StAR protein was detected by enhanced chemiluminescence using horseradish peroxidase conjugated secondary antibody and analyzed by densitometry. Blots were subsequently exposed to a second primary antibody directed against β-actin (1:1500; Chemicon, Temecula, CA) to verify equivalent protein loading. Western blot analysis shows that ethanol elicits a substantial increase in 30 kDa StAR protein expression in cytosolic and mitochondrial fractions. Representative blots for StAR protein and β-actin from saline (S) and ethanol (E) pre-treated rats are shown above for the respective fractions. (B) Ethanol exposure also increased plasma pregnenolone, progesterone and corticosterone. These values are presented as mean ± S.E.M. (ng/ml). The average plasma ethanol level in ethanol-injected rats was 236.5 mg/dl. *$P<0.001$ as compared to respective controls (Students t-test).

analysis using rabbit StAR antibody (1:1000, Calbiochem, San Diego, CA). Plasma pregnenolone, progesterone and corticosterone were assayed using radioimmunoassay kits (ICN Biochemicals, Costa Mesa, CA). Plasma ethanol levels were determined via the Analox GL-5 (Analox Instruments USA, Lunenburg, MA).

As depicted in Fig. 1A, ethanol (2 g/kg, i.p.) significantly increased StAR protein (30 kDa) expression in both cytosolic and mitochondrial fractions of rat adrenal glands. StAR peptide levels were increased 117.8% in the adrenal cytosolic fraction where new protein synthesis occurs. Furthermore, there was also a 115.2% increase in adrenal mitochondrial StAR, indicating that newly synthesized protein was transported to the mitochondria. Since StAR protein rapidly mediates the transfer of cholesterol from the outer to the inner mitochondrial membrane for pregnenolone biosynthesis, we measured plasma pregnenolone, progesterone and corticosterone. Ethanol administration coordinately increased pregnenolone (119 fold), progesterone (14 fold) and corticosterone (17 fold) (Fig. 1B).

These results are the first demonstration that acute systemic ethanol administration increases StAR protein expression in rat adrenal gland to elevate plasma pregnenolone, progesterone and corticosterone. Furthermore, elevated StAR protein expression in both the cytosolic and mitochondrial fractions suggests that newly synthesized StAR protein in the cytoplasm translocates to the mitochondria. The rapid transport of newly synthesized StAR protein (as opposed to total StAR) stimulates steroidogenesis by mediating cholesterol transfer to cytochrome P450scc in the inner mitochondrial membrane (Artemenko et al., 2001).

Although ethanol might influence other steroidogenic enzymes involved in neuroactive steroid synthesis, the elevation of StAR expression in the adrenals elevates pregnenolone levels, the first and rate-limiting step in the generation of these steroids. In ovaries, ethanol administration is reported to inhibit StAR expression and reduce serum pregnenolone and estradiol in prepubertal female rats (Srivastava et al., 2001).

Ethanol-induced elevation of GABAergic neuroactive steroids contributes to its behavioral effects including anticonvulsant, sedative-hypnotic, antidepressant and cognitive impairment (for a review see Khisti et al., 2002). These effects are all prevented by inhibition of steroidogenesis or adrenalectomy. Acute ethanol exposure increases the expression and translocation of adrenal StAR protein representing the mechanism of ethanol-induced elevation of neuroactive steroids. This mechanism may be relevant to the effects of stress and other psychoactive drugs that alter CNS excitability via synthesis of neuroactive steroids. It also provides potential new targets for drug intervention and alcoholism therapy.

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