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Hormonally regulated $\alpha_4\beta_2\delta$ $GABA_A$ receptors are a target for alcohol

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Here we report that low concentrations of alcohol (1–3 mM) increased Cl^- currents gated by a recombinant $GABA_A$ receptor, $\alpha_4\beta_2\delta$, by 40–50% in *Xenopus laevis* oocytes. We also found greater hippocampal expression of receptors containing α_4 and δ subunits, using a rat model1 of premenstrual[2] syndrome (PMS) in which 1–3 mM alcohol preferentially enhanced GABA-gated currents, and low doses of alcohol attenuated anxiety and behavioral reactivity. The alcohol sensitivity of δ-containing receptors may underlie the reinforcing effects of alcohol during PMS, when eye saccade responses to low doses of alcohol are increased[2].

Alcohol is an addictive recreational drug that reduces anxiety at low doses and causes sedation at high doses[3]. These effects are similar to those of drugs that enhance the function of $GABA_A$ receptors, which gate the Cl^- currents that mediate most inhibitory neurotransmission in the brain. Acutely high doses of alcohol potentiate GABA-gated currents[3] at both native[3] and recombinant $GABA_A$ receptors[4], and chronically alter $GABA_A$ receptor expression[5]. Low doses of alcohol have not been shown to directly modulate recombinant $GABA_A$ receptors[3], although there is indirect evidence for such effects at native receptors[6,7].

It has been suggested that discrepancies between the alcohol sensitivity of native and recombinant receptors may be due to their subunit composition[1]. We therefore investigated the effects of low concentrations of alcohol on different subtypes of recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes. Low (1 mM) concentrations of alcohol selectively increased GABA-gated currents at $\alpha_4\beta_2\delta$ receptors by 45 ± 11% ($P < 0.05$, Fig. 1a). Substitution of γ_2 for the δ subunit abolished this GABA-modulatory effect of alcohol (Fig. 1a), as did substitution of α_1 for α_4 when co-expressed with $\beta_2\delta$ (Fig. 1b). Furthermore, none of the other subunit combinations that yielded functional receptors (Supplementary Methods online) were

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sensitive to low concentrations of alcohol (Fig. 1a and b). Thus, co-expression of α_4 and δ subunits is necessary for potentiation by 1 mM alcohol.

The $\alpha_4\beta\delta$ $GABA_A$ receptors are expressed at very low levels in most regions of the brain[8]. Predicting that physiological states that are associated with increased sensitivity to alcohol (such as PMS) may involve increased expression of $\alpha_4\beta\delta$, we used a rodent model of PMS[1] to test this hypothesis. With chronic *in vivo* administration and withdrawal of progesterone, we replicated the hormonal and behavioral facets of PMS[2]. Expression of δ and α_4 subunit proteins in the hippocampus was three-fold higher after progesterone withdrawal (Fig. 2a and b, and Supplementary Fig. 1), as was δ-subunit mRNA (Supplementary Fig. 1). Co-assembly of these subunits was determined using co-immunoprecipitation (Fig. 2c) and verified by an increased efficacy of THIP, a GABA partial agonist, relative to GABA after progesterone withdrawal (Fig. 2d). This is characteristic of $\alpha_4\beta\delta$ receptor expression[9]. Co-assembly of β_2 with $\alpha_4\delta$ has not been tested directly but is suggested by reports of high levels of β_2 in areas rich in α_4 and δ subunits.

Concomitant with upregulation of $\alpha_4\beta\delta$ receptors in the progesterone-withdrawal model, 1 mM alcohol produced a 81 ± 21% potentiation of GABA-gated currents recorded from hippocampal neurons *in vitro* (Fig. 3a). Higher concentrations (10 mM and greater) of alcohol were not as effective in potentiating GABA-gated currents. After progesterone withdrawal, low doses of alcohol (0.2–0.4 g/kg) administered *in vivo* also decreased the acoustic startle response, a measure of behavioral excitability, suggesting a greater anxiolytic effect of alcohol at this time (Fig. 3b).

To verify that native $\alpha_4\beta\delta$ receptors were sensitive to low doses of alcohol, we repeated the progesterone withdrawal experiments after suppressing α_4 subunit expression. We have previously shown that, following progesterone withdrawal, injection of a GABA modulator prevents expression of the α_4 subunit[1]. Thus, we used alcohol here to suppress expression of the α_4 protein following progesterone withdrawal (P Wd + Alc; Fig. 2b and Supplementary Fig. 2). Under these conditions, the GABA-modulatory effects of 1 mM alcohol were prevented in hippocampal neurons (Fig. 3a; P Wd + α_4 suppressed), again implicating $\alpha_4\beta\delta$ receptors as sensitive to low doses of alcohol. There is also corroborating evidence that the reinforcing and anti-convulsant properties of alcohol are reduced in transgenic mice lacking the δ subunit[10].

It is thought that $\alpha_4\beta\delta$ receptors are expressed at extrasynaptic sites[8] where they may dampen neuronal excitability, primarily by acting as a resistive shunt[11]. Enhanced function of these receptors by low concentrations of alcohol in women with PMS would further decrease neuronal excitability, leading to behavioral stress-reduction. Blood alcohol levels of 1–3 mM may result from consumption of a half glass of wine[12] or less[13]. The reported increase in alcohol consumption and propensity for alcoholism in women with PMS[14] may thus be accounted for by these enhanced reinforcing properties of alcohol. More broadly, it is conceivable that alterations in $GABA_A$ receptors, perhaps including $\alpha_4\beta_2\delta$, are involved in the genetic predisposition for alcoholism in which there is an increased sensitivity to low doses of alcohol[15].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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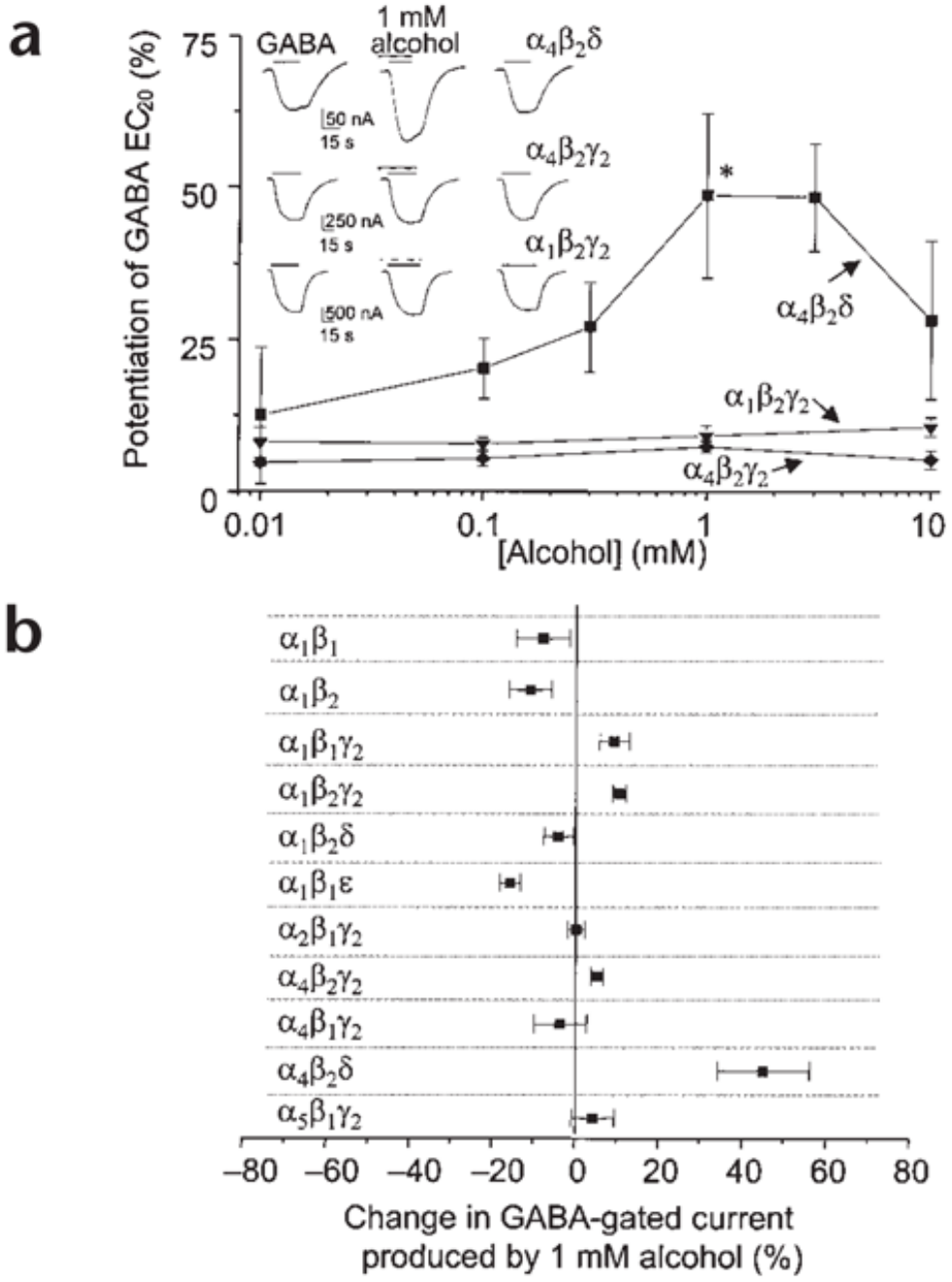

Fig. 1.

Low concentrations of alcohol potentiate GABA responses at $\alpha_4\beta_2\delta$ receptors. (**a**) The effects of alcohol on responses to GABA (EC_{20} = 0.03 μM $\alpha_4\beta_2\delta$, 1.7 μM $\alpha_1\beta_2\gamma_{2s}$, 5.6 μM $\alpha_4\beta_2\gamma_{2s}$) were measured by two-electrode voltage-clamp recording at −70 mV in oocytes expressing recombinant $GABA_A$ receptors. Inset, representative traces showing currents activated by GABA in the absence and presence of 1 mM alcohol. (**b**) Effects of 1 mM alcohol on GABA (EC_{20})-gated currents at various $GABA_A$ receptor subtypes (n = 8–20, *P < 0.05). Experiments were conducted according to Institutional Animal Care and Use Committee guidelines.

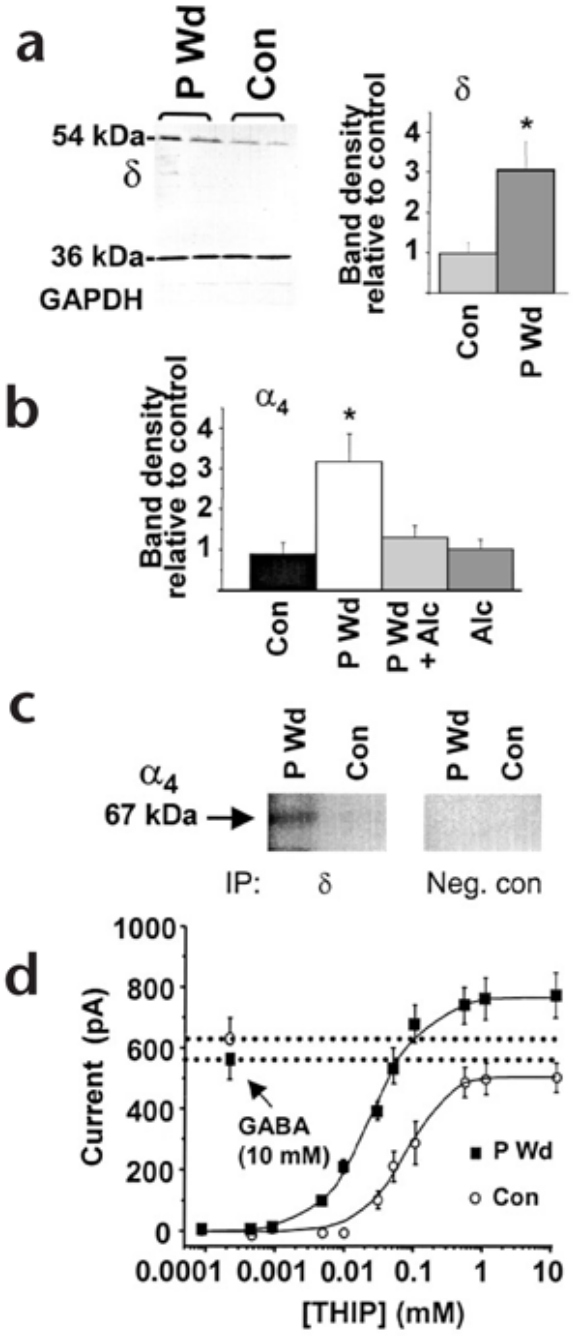

Fig. 2.

Progesterone withdrawal increases $\alpha_4\beta\delta$ $GABA_A$ receptors. (**a**) Left, western blot showing increased expression of the δ subunit (54 kDa), but not a control protein (GAPDH, 36 kDa) after progesterone withdrawal (P Wd) compared to control (Con). Right, mean values (n = 20–25, performed in triplicate). (**b**) Increases in α_4 protein following P Wd were prevented by *in vivo* administration of alcohol (0.5 g/kg × 3, intraperitoneally) during the final two hours of the withdrawal period (P Wd + Alc; n = 9–10). (**c**) Co-assembly of α_4 and δ $GABA_A$ receptor subunits. After immunoprecipitation (IP) using protein A beads coupled to antibodies for the δ subunit (IP, δ) or a cytosolic protein (IP, Neg. con) membranes were probed with digoxygenin-labeled anti-α_4 on a western blot (n = 4 hippocampi, in duplicate). A prominent

67-kDa band was detected after P Wd, but was barely detectable under control conditions. (**d**) The maximum current produced by THIP compared to that produced by 10 mM GABA was 1.41 after P Wd (THIP EC_{50} = 39 ± 2 μM), and 0.95 in control neurons (THIP EC_{50} = 81 ± 6 μM). This was determined using whole-cell patch clamp recording in neurons from CA1 hippocampus (n = 10–15, *P < 0.05).

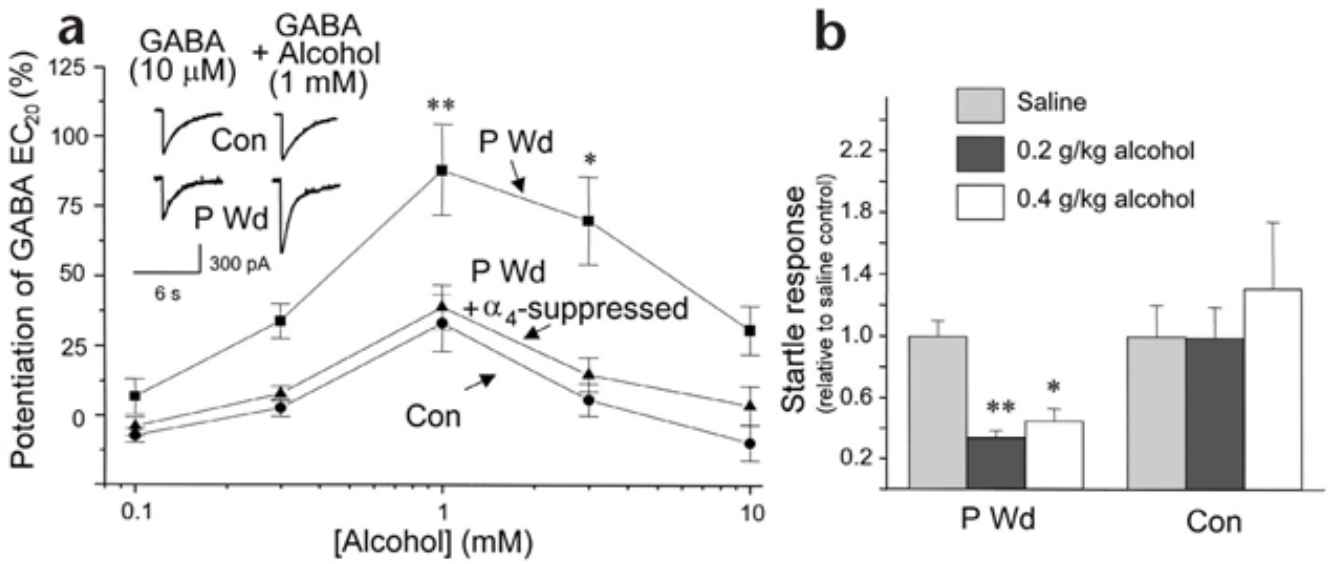

Fig. 3.

Low doses of alcohol potentiate GABA-gated currents in hippocampal pyramidal neurons and decrease behavioral excitability after progesterone withdrawal. (**a**) GABA (10 μM)-gated currents recorded in the presence or absence of alcohol (0.1–10 mM), measured by whole-cell patch clamp recording after progesterone withdrawal (P Wd) or under control conditions (con). Inset, representative traces. Suppression of α_4 subunit expression (P Wd + α_4 suppressed), as described in Fig. 2b, abolished the stimulatory effects of low concentrations of alcohol after P Wd (n = 20–25 cells/concentration, 8–10 rats/group). (**b**) Low doses of alcohol administered to P Wd (but not control) rats significantly lowered the acoustic startle response, expressed relative to the saline control (n = 5–11, *P < 0.05, **P < 0.005).