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Terminal Effects of Ethanol on Dopamine Dynamics in Rat Nucleus Accumbens: An In Vitro Voltammetric Study

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ABSTRACT To assess the direct effects of acute ethanol on dopamine (DA) terminals, evoked DA release and uptake were measured in rat nucleus accumbens slices using fast-scan cyclic voltammetry. Low and moderate concentrations of ethanol (20, 45 and 100 mM) did not alter evoked DA release, while high concentrations (150 and 200 mM) significantly decreased DA release (18 and 36%, respectively) in a calcium-dependent manner. No significant difference was found between the rate of DA disappearance measured before and after the drug. These data indicate that uptake of DA through the dopamine transporter is unaffected by ethanol, even at high concentrations. Therefore, low to moderate concentrations of ethanol have no effect on DA dynamics at the level of the nerve terminal in the nucleus accumbens. This is consistent with the hypothesis that cell body regions of DA neurons are the primary target for the stimulating and reinforcing effects of ethanol. High concentrations of ethanol can locally depress DA release, and this may correlate with the sedative actions of the drug. **Synapse 42:77–79, 2001.**

Despite a large diversity in mechanisms of action, addictive drugs share the ability to increase extracellular dopamine (DA) levels in the nucleus accumbens (Berke and Hyman, 2000). At low doses, ethanol shares the DA-enhancing properties of other abused drugs (Imperato and Di Chiara, 1986; Blanchard et al., 1993; Yan, 1999; Yim et al., 2000). However, at high doses ethanol can suppress DA levels (Imperato and Di Chiara, 1986; Blanchard et al., 1993; but see also Mocsary and Bradberry, 1996; Yan, 1999). These biphasic neurochemical changes suggest that ethanol can facilitate or inhibit mesolimbic DA transmission, depending on the dose. Different mechanisms are probably responsible for the two effects. The facilitation is consistent with ethanol causing an increase in the firing rate of DA neurons in the ventral tegmental area (VTA) (Brodie et al., 1990, 1999), although terminal effects have not been ruled out. Ethanol-mediated DA neuron excitation may occur directly at DA cell bodies (Brodie et al., 1999) or indirectly; for example, via a decrease in the firing rate of VTA GABAergic interneurons (Gallegos et al., 1999). The mechanism of the inhibitory influence of ethanol on mesolimbic DA is less clear.

Recently, using fast-scan cyclic voltammetry (FSCV) to rapidly sample extracellular DA in slices of rat caudate-putamen, we found that high concentrations of ethanol decreased stimulated DA release (Budygin et al., 2001). Since the cell bodies and most of the neuronal pathways are removed in this preparation, these data suggest that inhibitory mechanisms could take place at the terminal level. However, the facilitatory effects of ethanol on DA at lower doses clearly do not involve DA terminals in the caudate-putamen, since no change in release or uptake was observed at these doses. To determine whether the same is true in the nucleus accumbens, a more likely site of reinforcing ethanol effects, in the present study we evaluated the effects of low and high concentrations of ethanol on DA transmission in slices of the nucleus accumbens.

Male Sprague-Dawley rats (200–250 g) were sacrificed by decapitation and the brains rapidly removed and cooled in ice-cold, pre-oxygenated (95% O_2/5% CO_2), modified Kreb's buffer. The tissue was then sectioned into 400 μm-thick coronal slices containing the nucleus accumbens, using a vibrating tissue slicer

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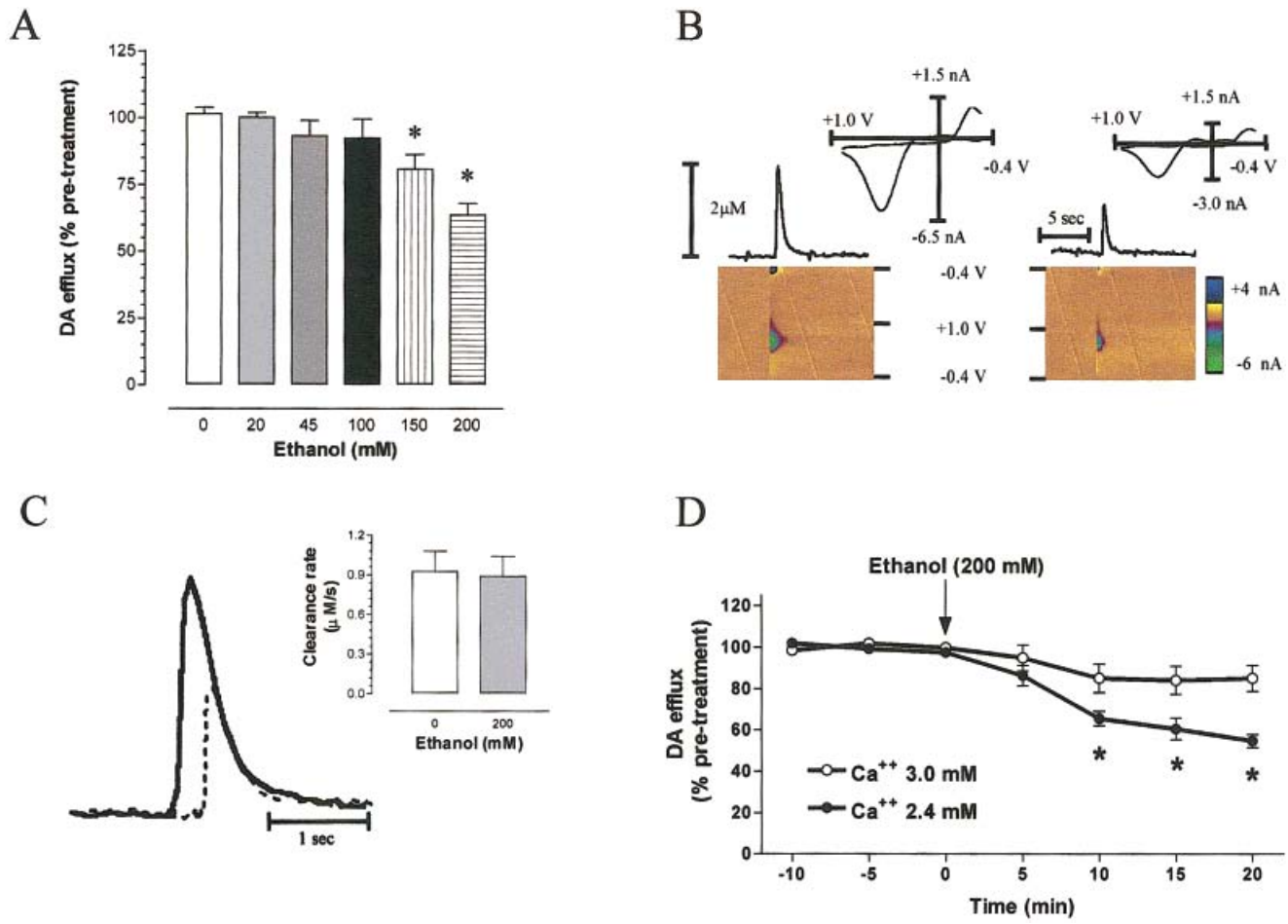

Fig. 1. Effect of ethanol on locally evoked DA efflux in the nucleus accumbens. **A:** Locally evoked DA overflow measured by FSCV in slices during ethanol bath application (20 min). Significant values for DA efflux, expressed as % pre-treatment, are as follows: 150 mM, 81.8 ± 5.6; 200 mM, 63.7 ±4.2; $^*P < 0.05$ versus time-matched control value (101 ± 2.5), $n = 4$ or 5 animals per group. **B:** Representative color plots, concentration-time plots, and cyclic voltammograms of DA before (left) and after (right) 200 mM ethanol in the same slice. The color plot topographically depicts the voltammetric data, with time on the x-axis, applied scan potential on the y-axis (scan direction from bottom to top) and background-subtracted faradaic current measured on the z-axis in pseudocolor. Directly above the color plot is the faradaic current (solid line) at the oxidation potential for DA extracted from the color plot, depicted so that an increase in extracellular DA would produce an upward deflection. At the top is a background-subtracted cyclic voltammogram taken at the peak response. This has an oxidation peak at 600 mV and a reduction peak at −200 mV versus Ag/AgCl reference, identifying the released species as DA. For further explanation of this type of data representation, see Michael et al. (1998). **C:** Electrically stimulated DA overflow measured by FSCV in slices before (solid line) and during 200 mM ethanol bath application (20 min, dashed line). The curves are arranged so that the descending portion of the curves are aligned in order to show that the clearance rate of DA by the DA transporter is identical in the presence or absence of ethanol. There was no change in clearance rate at any dose of ethanol. **Inset:** Clearance rate of evoked DA in μM/s, measured from the peak amplitude of the postdrug response to baseline on both pre- and postdrug curves. **D:** Locally evoked DA overflow in nucleus accumbens slices before and during 200 mM ethanol ($n = 4$ animals per group) bath application (20 min) in the presence of 2.4 or 3.0 mM calcium. Data are plotted as mean ± SEM. $^*P < 0.05$ vs. 3.0 mM calcium values.

(Leica VT1000S, Leica Instruments, Germany or Vibroslice HA752, Campden Instruments, UK). Slices were kept in a reservoir of oxygenated Kreb's at room temperature until required. Thirty minutes before each experiment, a brain slice was transferred to a "Scottish-type" submersion recording chamber, perfused at 1 ml/min with 34°C oxygenated Kreb's, and allowed to equilibrate. The Kreb's buffer consisted of (in mM): NaCl (126), KCl (2.5), NaH_2PO_4 (1.2), $CaCl_2$ (2.4), $MgCl_2$ (1.2), $NaHCO_3$ (25), glucose (11), HEPES (20), L-ascorbic acid (0.4), and pH adjusted to 7.4. DA was evoked by a single, rectangular, electrical pulse (300 μA, 2 ms/phase, biphasic) applied every 5 min. DA was detected using FSCV as described previously (Budygin et al., 2001). Once the extracellular DA response to electrical stimulation was stable for three successive stimulations, ethanol (0, 20, 45, 100, 150, or 200 mM) was applied to the nucleus accumbens via the superfusate. All statistical analyses were carried out using GraphPad Prism (GraphPad Software, San Diego, CA).

The experiments indicated that evoked DA efflux in the nucleus accumbens is affected by ethanol in a manner similar to that observed in the caudate-putamen (Budygin et al., 2001). High concentrations of ethanol

decreased stimulated DA efflux, while low concentrations did not alter DA efflux (Fig. 1A,B). In addition, ethanol did not induce any changes in DA uptake, as measured by the rate of DA disappearance after stimulation (Fig. 1C). Thus, at the low concentrations of ethanol often examined for reinforcing and intoxicating effects, there are no changes in DA dynamics at nerve terminals in the nucleus accumbens that could explain the DA-enhancing effects of ethanol.

Since the amplitude of the evoked DA efflux measured in the present experiments by voltammetry is a function of exocytotic DA release, decreases in the amplitude induced by high concentrations of ethanol must be due to altered release. Therefore, the decreased extracellular DA measured by microdialysis following administration of high doses of ethanol (Imperato and Di Chiara, 1986 [5 g/kg]; Blanchard et al., 1993 [2 g/kg]) is at least partly a consequence of depressed release. Because the cell bodies are removed in the slices, this effect of ethanol must be mediated at the level of the presynaptic terminals.

One of the mechanisms through which high concentrations of ethanol might decrease evoked DA efflux is by reducing the rate of biosynthesis. However, moderate and high doses of ethanol actually increase DA synthesis, as measured by the accumulation of tissue levels of L-DOPA following inhibition of aromatic acid decarboxylase (Carlsson and Lindquist, 1973; Inoue, 2000; Budygin et al., 2001). This effect of ethanol might be due to decreased DA levels following administration of high doses. Lower extracellular DA levels would lead to relief of inhibition from synthesis-modulating autoreceptors on the DA nerve terminals and thereby increase synthesis rates.

Ethanol has a multitude of actions that may lead to DA release inhibition at the level of the nerve terminal, including effects on receptors and ion channels (Crews et al., 1996). We tested the presynaptic nature of the decrease in DA release by increasing the concentration of calcium in the perfusion buffer. Ethanol was significantly less effective in decreasing DA release when the calcium concentration was increased from 2.4 to 3.0 mM (Fig. 1D). This result shows that increased calcium influx can overcome the inhibitory effects of ethanol on DA release and suggests that ethanol works by decreasing calcium influx, either directly or indirectly.

Taken together with previous reports (Budygin et al., 2001), these data indicate that low concentrations of ethanol have no effect on DA release or uptake at the level of the nerve terminal in the nucleus accumbens. High concentrations of ethanol (150–200 mM) can locally depress DA release and this effect may correlate with the sedative actions of the drug and perhaps play a role in its toxic effects. The dose-dependent, biphasic effects of ethanol on the DA system seem to be caused by differential sensitivity of cell body regions and presynaptic terminals to ethanol. Decreased extracellular DA induced by high doses of ethanol can be explained, at least in part, by direct terminal effects in the nucleus accumbens. However, it seems clear that the well-documented elevation in extracellular DA at low doses is not due to terminal effects, but more likely to changes in firing rate at the level of the cell bodies.

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