The Effects of Long Chain-Length n-Alcohols on the Firing Frequency of Dopaminergic Neurons of the Ventral Tegmental Area

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ABSTRACT

The dopaminergic neurons of the ventral tegmental area (DA VTA neurons) have been implicated in the reinforcing properties of drugs of abuse, including ethanol (ethyl alcohol). Ethanol increases the spontaneous firing frequency of DA VTA neurons in vitro, in both brain slices and acutely dissociated neurons, and also in vivo. In many systems, longer n-alkyl alcohols have a more potent effect than ethanol, and the potency is a function of the number of carbons in the alkyl chain. We studied n-alcohols of chain length 1 (methanol) to 5 (pentanol) on the firing rate of DA VTA neurons in brain slice preparations. All of the alcohols studied produced increases in the spontaneous firing frequency in DA VTA neurons; as the chain length increased, lower concentrations of the alcohols were needed to produce the same percentage increase in firing. With very high concentrations of all the alcohols except methanol, we observed apparent depolarization block of firing. In addition, trichloroethanol (TCE), the active metabolite of chloral hydrate, increased the firing frequency of DA VTA neurons, and the EC40 (concentration to produce a 40% increase in firing rate) of TCE was below that of ethanol. These studies indicate that excitation of VTA dopamine neurons by n-alcohols is related to the chain length of the carbons. This is likely to be a characteristic of the ethanol-sensitive element of DA VTA neurons and may be useful in identifying the element of the membrane that is responsible for ethanol-induced excitation.

Dopaminergic (DA) neurons originating in the ventral tegmental area (VTA) provide the DA innervation to the nucleus accumbens and prefrontal cortex and therefore are the source of the mesolimbic and mesocortical pathways (Oades and Halliday, 1987). Behavioral and pharmacological data indicate that the mesolimbic dopamine pathway is important in the reinforcing effects of ethanol. Oral self-administration and systemic injection of ethanol increase dopamine release in the nucleus accumbens (Di Chiara and Imperato, 1988; Weiss et al., 1993). The role of dopamine in ethanol reinforcement is further supported by pharmacological studies with DA agonists and antagonists (Samson et al., 1990). Although dopamine antagonists do not block or reverse all of the behaviors related to the rewarding properties of ethanol (Cunningham et al., 1992; Risinger et al., 1992), it has been shown recently that dopamine antagonists can reduce ethanol reward (Price and Middaugh, 2004) and consumption (Thanos et al., 2005) in some paradigms in rodents. In addition, rats will self-administer ethanol directly into the VTA (Rodd-Henricks et al., 2000). Ethanol excitation of dopamine cell bodies in the VTA in vivo (Gessa et al., 1985) would result in increased release of dopamine from terminals in the nucleus accumbens. Ethanol also excites DA VTA neurons in brain slices, and this effect persists when Ca2+-dependent synaptic transmission is blocked with low Ca2+/high Mg2+ media (Brodie et al., 1999). Furthermore, we have shown that ethanol excites acutely dissociated DA VTA neurons that have been isolated from all of the synaptic inputs, providing conclusive evidence that ethanol directly excites DA VTA neurons (Brodie et al., 1999b).

Many studies have shown that longer chain-length alcohols have effects on biological systems qualitatively similar to the effect of ethanol (Wallgren, 1960; McCreey and Hunt, 1978; Lyon et al., 1981). In general, the actions of longer chain-length alcohols are observed at lower concentrations than those needed to observe ethanol actions (Franks and Lieb, 1985, 1986; Peoples and Weight, 1995). Many actions of

ABBREVIATIONS: DA, dopaminergic; VTA, ventral tegmental area; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; TCE, trichloroethanol; AP, action potential.
alcohol are believed to occur by the interaction of alcohol with proteins in the membrane, either ion channels (Anantharam et al., 1992; Dopico et al., 1996) or neurotransmitter receptors (Allan and Harris, 1987; Løvinger, 1990; Løvinger et al., 1990).

Although it is clear that ethanol increases the spontaneous activity of DA VTA neurons, the effect of longer chain-length alcohols has not been characterized. The present study examines the action of longer chain-length n-alcohols on the spontaneous firing rate of DA VTA neurons. No study of longer chain alcohols has been carried out on DA VTA neurons to date. The literature indicates that in rats, the intoxicating effects of alcohols increase with chain length to at least butanol (Wallgren, 1960). This was also observed by Lyon et al. (1981), who showed that the membrane-disordering properties and the hypnotic potency of alcohols increased with increasing chain length of n-alcohols. The effect of alcohols to produce ataxia was also dependent on chain length, with longer chain-length alcohols producing ataxia more potently (McCreery and Hunt, 1978). These data and anecdotal evidence of the potency of longer chain-length alcohols consumed by humans suggest that, like ethanol, longer chain-length alcohols may increase the firing rate of DA VTA neurons and do so with higher potency.

In addition, to make a more complete comparison with the longer chain-length alcohols, concentrations of ethanol were tested that exceeded the concentration range that has been tested in our laboratory in the past. Information on the effect of longer chain-length alcohols is critical to characterize the ethanol-sensitive elements of DA VTA neurons and is necessary for the identification of the ion channels involved in ethanol-induced excitation of DA VTA neurons.

Materials and Methods

Preparation of Brain Slices. Brain slices containing the VTA were prepared from male Fischer-344 rats (90–150 g) as described previously (Appel et al., 2000). Animals used in this study were treated in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and all of the experimental methods were approved by the Animal Care Committee of the University of Illinois at Chicago. In brief, after rapid removal of the brain, the tissue was blocked coronally to contain the VTA and substantia nigra; the cerebral cortices and a portion of the dorsal mesencephalon were removed. The tissue block was mounted in a vibratome and submerged in chilled artificial cerebrospinal fluid (aCSF). Coronal sections (400 μm thick) were cut, and the slice was placed onto a mesh platform in the recording chamber. The slice was totally submerged in aCSF maintained at a flow rate of 2 ml/min; the temperature in the recording chamber was kept at 35°C. The composition of the aCSF in these experiments was 126 mM NaCl, 2.5 mM KCl, 1.24 mM NaH2PO4, 2.4 mM CaCl2, 1.3 mM MgSO4, 26 mM NaHCO3, and 11 mM glucose. The aCSF was saturated with 95% O2/5% CO2 (pH 7.4). Equilibration time of at least 1 h was allowed after placement of tissue in the recording chamber before electrodes were placed in the tissue. The VTA was clearly visible in the fresh tissue as a gray area medial to the darker substantia nigra and separated from the nigra by white matter. Recording electrodes were placed in the VTA under visual control. Dopamine neurons have been shown to have electrophysiological characteristics very different from non-DA neurons in the mesencephalon (Lacey et al., 1989).

Only those neurons that were anatomically located within the VTA and that conformed to the criteria for DA neurons established in the literature and in this laboratory (Lacey et al., 1989; Mueller and Brodie, 1989) were studied. These criteria include broad action potentials and slow spontaneous firing rate (0.5–5 Hz) with a regular interspike interval.

Drug Administration for Brain Slices. Drugs were added to the aCSF by means of a calibrated infusion pump from stock solutions 100 to 1000 times the desired final concentrations. The addition of drug solutions to the aCSF was performed in such a way as to permit the drug solution to mix completely with the aCSF before this mixture reached the recording chamber. The use of a calibrated, variable-speed infusion pump permits the accurate addition of several concentrations of drug from the same stock solution. Final concentrations were calculated from the aCSF flow rate, pump infusion rate, and concentration of drug stock solution. The small volume chamber (~300 μl) used in these studies permitted the rapid application and washout of drug solutions. Typically drugs reached equilibrium in the tissue after 2 to 3 min of application. We have used this method reliably in the past, and it offers the advantage of being able to test numerous concentrations of the test drugs without the possible confounding variable of slight differences in the composition (pH and salt concentration) of the aCSF. We are able to change the concentration of the drugs over an approximately 10-fold range very discretely and consistently.

For ethanol, a stock solution of 95% ethanol (v/v USP) was used in the pump, and infusion of ethanol never exceeded 1% of the flow rate of the aCSF. For other alcohols (greater than 99% v/v), the infusion also was less than 1% of the flow rate of the aCSF, with the exception of methanol, and the highest concentrations of methanol that were tested were less than 2% of the flow rate of the aCSF. Alcohols were administered for 5 to 7 min to ensure that measurements were made after the full alcohol concentration was reached in the tissue and the peak drug effect was attained. In cases in which alcohols caused cessation of firing, alcohol infusion was halted after the firing had stopped for 30 s. The values for concentration reported below reflect the calculated concentrations of the alcohols tested. Because of volatilization of alcohols, the real concentrations may be below these exact values. However, because of the relatively high rate of inflow of medium (2 ml/min) compared with the recording chamber volume (approximately 300 μl), we are confident that the concentrations reported below are close to the final bath concentrations.

The behaviorally active range for blood ethanol concentrations in the rat extends from 40 (sedation) to 90 mM (loss of righting reflex) (Majchrowicz and Hunt, 1976); the lethal blood ethanol concentration in rats is approximately 200 mM (LD50 = 202 mM) (Haggard et al., 1940). Rats will self-administer 44 to 55 mM ethanol directly into the VTA, indicating that this concentration is reinforcing in the whole animal (Rodd-Henricks et al., 2000). As we observed apparent depolarization blockade of firing with some of the longer chain-length alcohols and we had not observed this phenomenon in previous studies in which the concentrations ranges were closer to the pharmacologically relevant concentrations in rats (Brodie et al., 1990; Brodie and Appel, 1998), the present study examined ethanol concentrations above the concentration that would be lethal in the intact rat for adequate comparison with longer chain-length alcohols.

Extracellular Recording in Brain Slices. Extracellular recording electrodes were made from 1.5-mm diameter glass tubing with filament and were filled with 0.9% NaCl. Tip resistance of the microelectrodes ranged from 3 to 8 MΩ. The Finitronics amplifier (Finitronics, Inc., Orange, CT) used in these recordings includes a window discriminator, the output of which was fed to both a rectilinear pen recorder, and a computer-based data acquisition system, which was used for on-line and off-line analysis of the data. The multiplexed output of the Finitronics amplifier was displayed on an analog storage oscilloscope for accurate adjustment of the window levels used to monitor single units. A personal computer-based data acquisition system was used to calculate, display, and store the frequency of firing over 5-s and 1-min intervals. Firing rate was determined before and during drug application. Firing rate was calculated over a 1-min interval immediately before drug administration and a 1-min interval during the peak drug effect; drug-induced changes in...
firing rate were expressed as the percentage change from the control firing rate according to the formula \([\frac{FR_{D} - FR_{C} \times 100}{FR_{C}}]\), where \(FR_{D}\) is the firing rate during the peak drug effect and \(FR_{C}\) is the control firing rate. Thus, the change in firing rate is expressed as a percentage of the initial firing rate, which controls for small changes in firing rate that may occur over time.

**Preparation of Dissociated Cells.** Fischer-344 rats (14–18 days old) were decapitated, and the brain was quickly removed. The brain was placed in ice-cold cutting solution (120 mM sucrose, 2.5 mM KCl, 2.4 mM CaCl_2, 1.3 mM MgSO_4, 1.24 mM NaH_2PO_4, 26 mM NaHCO_3, and 11 mM d-glucose), which was constantly bubbled with 95% O_2 and 5% CO_2. Transverse brain slices (350–400 \(\mu\)m) were made on a Vibratome (Series 1000 plus; St. Louis, MO). Brain slices were incubated for 30 min in aCSF (126 mM NaCl, 2.5 mM KCl, 2.4 mM CaCl_2, 1.3 mM MgSO_4, 1.24 mM NaH_2PO_4, 26 mM NaHCO_3, and 11 mM d-glucose; osmolality, 300 mOsm), which was constantly bubbled with 95% O_2 and 5% CO_2 at room temperature (23–25°C). The brain slices were then incubated in a HEKES-buffered solution (145 mM NaCl, 2.5 mM KCl, 2 mM CaCl_2, 1 mM MgCl_2, 10 mM HEPES, and 11 mM d-glucose, pH 7.4 adjusted with NaOH; osmolality, 300 mOsm) containing papain (15–18 U/ml) (Worthington, Lakewood, NJ) at 32°C for 20 to 25 min. After papain treatment, the brain slices were further incubated in aCSF for 20 to 40 min. VTA neurons were dissociated with a vibrating stylus apparatus for dispersing cells from the brain slices as described previously (Koyama et al., 2005).

**Drug Administration for Dissociated Cells.** Neurons were continuously bathed in control HEKES-buffered solution, and drugs were dissolved at final concentration in the same solution. Drug solutions were applied via a multiple channel manifold (ALA Scientific Instruments, Westbury, NY). Each channel of the manifold was connected to a gravity-fed reservoir with tubing (860 \(\mu\)m i.d.). The tip of which was placed within 100 \(\mu\)m of the soma of the recorded neuron. Solution flowed continuously through one manifold channel. Application of drug solutions was controlled by opening or closing valves connected to the reservoirs.

**Nystatin-Perforated Patch Recording in Dissociated Cells.** Electrophysiological measurements were made with an Axopatch-1B patch-clamp amplifier (Axon Instruments, Union City, CA). Microelectrodes were fabricated on a P-87 puller (Sutter Instrument Company, Novato, CA) from LE16 glass capillaries (Dagan, Minneapolis, MN) and heat-polished on a microforge (Narishige, Tokyo, Japan). The tip resistances of the electrodes were 1.5 to 3 M\(\Omega\) when filled with pipette solution (60 mM potassium acetate, 60 mM KCl, 1 mM CaCl_2, 2 mM MgCl_2, 40 mM HEPES, pH 7.2 adjusted with KOH; final \([K^+]_o = 131\) mM; osmolality was adjusted to 290 mOsm with sucrose). Nystatin-perforated patch recording was used as described previously (Koyama and Appel, 2006). Current-clamp recording was done in the HEKES-buffered solution constantly bubbled with 100% O_2. The liquid junction potential between the pipette solution and the HEKES-buffered solution was estimated to be 5 mV (Neher, 1992), and the results have been corrected by this amount. Data acquisition was performed with a Digidata 1322A interface and pClamp software version 9.0 (Axon Instruments). The dissociated VTA neurons were visualized under phase-contrast optics on an inverted microscope (Diaphot 300; Nikon, Tokyo, Japan). All of the experiments were performed at room temperature (23–25°C).

**Statistical Analysis.** Averaged numerical values were expressed as the mean ± S.E.M. The significance of firing rate changes before and after a single drug concentration was assessed with a paired t test. For effects of multiple drug concentrations or more than one drug, an appropriate one- or two-way analysis of variance (ANOVA) was used, followed by Student Newman-Keuls post hoc comparisons when needed.

**Results**

Spontaneously active VTA neurons were recorded in a series of extracellular recording experiments. The mean firing rate for all of the cells recorded was 1.17 ± 0.08 Hz (\(n = 46\)). Complete recovery was observed after washout of each alcohol tested. In cases in which there was no recovery, the response of that cell to that concentration of alcohol was discarded.

**Excitation of DA Neurons of the VTA: Methanol through Pentanol.** The concentration-response relationship between alcohol concentration and change in the firing rate of DA VTA neurons was assessed for a number of \(n\)-alcohols of increasing carbon chain length. The \(n\)-alcohols from chain length 1 (methanol) to 5 (pentanol) produced concentration-dependent excitation of DA VTA neurons in brain slices.

To illustrate a comparison of the effects of \(n\)-alcohols of different chain lengths on DA VTA neuronal firing, examples of the effects of ethanol, butanol, and pentanol are illustrated in Fig. 1. In these experiments with individual DA VTA neurons, the excitatory effect of 120 mM ethanol (Fig. 1A), 11 mM butanol (Fig. 1B), and 4.6 mM pentanol (Fig. 1C) can be seen. In these examples, 120 mM ethanol increased the firing rate by 44.9%; 11 mM butanol increased the firing rate by 81.8%; and 4.6 mM pentanol increased the firing rate by 91.7%. The excitatory effects were clearly reversible with washout of the alcohols.

Figure 2 illustrates the concentration-response relationship for excitation of DA VTA neurons by \(n\)-alcohols that were observed in the population of neurons in this study. The concentration-response curves constructed from mean responses from experiments with pentanol (\(n = 13\)), butanol (\(n = 9\)), ethanol (\(n = 26\)), and methanol (\(n = 6\)) are shown in Fig. 2. The percent increase in firing rate was calculated as described under Materials and Methods from records such as those shown in Fig. 1. Error bars represent the mean ± S.E.M. Smooth curves are sigmoidal curves fit to data points.

**Effect of High Concentrations of Ethanol.** Previous studies from our laboratory indicated that ethanol increased the firing rate of DA VTA neurons in a monotonically increasing concentration-dependent manner at concentrations from 10 to 320 mM (Brodie et al., 1990; Brodie and Appel, 1998). In past studies, the ethanol concentration was limited to application of a maximum of 320 mM (Brodie et al., 1990), which was well above the lethal concentration in rats (Haggard et al., 1940). We decided to test concentrations of ethanol in excess of 320 mM to determine whether higher concentrations of ethanol continued to be excitatory, or whether the cells would make the transition into apparent depolarization blockade. A typical recording is shown in Fig. 3A. In this case, 360 mM ethanol was added to the superfusate, and an initial increase in firing rate was followed by an abrupt cessation of firing. On washout of ethanol, firing resumed, first at an elevated rate compared with pre-ethanol firing, and then the firing returned to the baseline firing rate. This type of response was observed in seven of seven cells tested with ethanol concentrations from 360 to 450 mM. In general, concentrations of ethanol greater than 400 mM would produce this phenomenon, which is likely to be depolarization blockade. Because these are extracellular recordings, we cannot be certain that it is, in fact, depolarization blockade, but this is
more likely than a sudden hyperpolarization, which occurs only at high ethanol concentrations.

**Effect of High Concentrations of Longer Chain Alcohols (Butanol to Pentanol).** High concentrations of longer chain alcohols also produced apparent depolarization block. The response of typical DA VTA neurons to bath application of 43.5 mM butanol (B) and 9.2 mM pentanol (C) is shown in Fig. 3. Note that like the response to high ethanol shown in Fig. 3A, the firing rate first increased and then ceased abruptly. This absence of firing lasted until the alcohol application was stopped. This block of firing may have been the result of depolarization block (action potential failure caused by sodium channel inactivation). When butanol or pentanol was washed out, firing resumed first at an elevated rate, and then it returned to baseline. Note that this apparent depolarization block phenomenon was seen with concentrations of these alcohols higher than those that caused pure excitatory responses in these neurons, concentrations at the highest end or above the range tested in the concentration-response curves shown in Fig. 2.

Apparent depolarization blockade phenomena were observed with all of the \( n \)-alcohols tested of carbon chain length 2 or greater. Because high alcohol concentrations produce the apparent depolarization blockade phenomenon, it is not possible to accurately assess the potency of these alcohols using firing rate as the measure because the asymptotic saturation of the excitatory effect cannot truly be measured. This makes comparison of the effects of the longer chain alcohols more difficult. However, the mean minimal concentration at which apparent depolarization block is observed can be compared for different alcohols.

**Excitation of DA Neurons of the VTA: Relation between Hydrophobicity and Potency.** There was a log-log linear relation between hydrophobicity and potency based on the \( EC_{40} \) of the effect of the alcohols on firing frequency. This is illustrated in Fig. 4A. The concentration of ethanol, butanol, and pentanol that would produce a 40% increase in firing rate was determined from the concentration-response curves in Fig. 1 and plotted on the y-axis as the \( EC_{40} \) (effective

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**Fig. 1.** Effect of alcohols on firing rate: data from single neurons. Extracellular single unit recordings of the firing rate of typical DA VTA neurons. Firing rate is plotted as a function of time; vertical bars are proportional to the mean firing rate over 5-s intervals. Horizontal bars indicate duration of application of ethanol (A), butanol (B), or pentanol (C). A, ethanol (120 mM) produced an increase in firing rate of 44.9%. B, butanol (11 mM) produced an increase in firing rate of 81.8%. C, pentanol (4.6 mM) produced an increase in firing rate of 91.7%. Note that the excitatory effects were clearly reversible with washout of the alcohols.

**Fig. 2.** Effect of alcohols on firing rate: pooled data. Pooled concentration-response curves for increases in firing rate produced by \( n \)-alcohols (methanol, ethanol, butanol, and pentanol) from experiments similar to those shown in Fig. 1. The data were pooled from cells tested with ethanol (\( n = 26 \)), butanol (\( n = 9 \)), pentanol (\( n = 13 \)), and methanol (\( n = 6 \)). The effects of each alcohol were concentration-dependent (two-way ANOVA, \( p < 0.05 \)).
concentration 40%). Methanol was not included because a 40% increase in firing rate was not achieved over the concentration range tested in this study. The equieffective concentrations of the three alcohols that produced a 40% increase in firing rate estimated from the concentration-response curves are pentanol (3.8 mM), butanol (10.4 mM), and ethanol (120 mM). The octanol/water partition coefficient, an index of hydrophobicity, of each alcohol was plotted on the $x$-axis. The partition coefficients were ethanol (0.096), butanol (1.52), and pentanol (5.02) (McCreery and Hunt, 1978). Note that both the $x$- and $y$-axes are on a logarithmic scale. The dotted line was determined by linear regression; the correlation coefficient ($R^2$ value) was 0.999.

**Fig. 3.** Effect of high concentrations of alcohols on firing rate: apparent depolarization block of single neurons. Extracellular single unit recordings of the firing rate of typical DA VTA neurons. Firing rate is plotted as a function of time; vertical bars are proportional to the mean firing rate over 5-s intervals. Horizontal bars indicate duration of application of 360 mM ethanol (A), 43.5 mM butanol (B), or 9.2 mM pentanol (C). Note that with the application of the alcohol, the firing rate first increases and then ceases abruptly. This absence of firing lasts until the alcohol application is stopped.

**Fig. 4.** Correlations between partition coefficient and alcohol effects. The EC$_{40}$ (A) and the minimal concentration needed to produce depolarization block (B) were plotted as a function of the octanol/water partition coefficient. The octanol/water partition coefficients, an index of hydrophobicity, used in these graphs are ethanol (0.096), butanol (1.52), and pentanol (5.02) (McCreery and Hunt, 1978). A, increase in firing rate (EC$_{40}$): the concentration of ethanol, butanol, and pentanol that would produce a 40% increase in firing rate was determined from the concentration-response curves in Fig. 2 and plotted on the $y$-axis as the EC$_{40}$ (effective concentration 40%). The equieffective concentrations of the three alcohols that produced a 40% increase in firing rate estimated from the concentration-response curves were pentanol (3.8 mM), butanol (10.4 mM), and ethanol (120 mM). The line was determined by linear regression; the correlation coefficient ($R^2$ value) was 0.999. B, apparent depolarization block: for each alcohol (ethanol, butanol, and pentanol) the concentration above which the neuron abruptly ceases firing was quantified. This was done by exposing cells to 12-min applications of the alcohol at increasing concentrations. The mean minimal concentration for producing this apparent depolarization block of firing was 12.3 ± 1.6 mM for pentanol ($n = 3$), 28.9 ± 1.4 mM for butanol ($n = 4$), and 360 ± 31 mM for ethanol ($n = 5$). The line was determined by linear regression; the correlation coefficient ($R^2$ value) was 0.997.
We also determined that there is a log-log linear relation between hydrophobicity of each alcohol and the minimal concentration of that alcohol that was required to produce apparent depolarization block of firing. To ensure complete equilibration of the high alcohol concentrations, 12-min applications of the alcohol at increasing concentrations were used. The results of these studies are illustrated in Fig. 4B. The minimal concentration of ethanol, butanol, and pentanol that would produce apparent depolarization block was determined in a series of experiments with protocols similar to those shown in Fig. 3, and plotted as a function of the octanol/water partition coefficient, an index of hydrophobicity. The minimal concentration for producing this apparent depolarization block of firing was 12.3 ± 1.6 mM for pentanol (n = 3), 28.9 ± 1.4 mM for butanol (n = 4), and 360 ± 31 mM for ethanol (n = 5). The partition coefficients are ethanol (0.096), butanol (1.52), and pentanol (5.02) (McCreery and Hunt, 1978). Note that both the x- and y-axes are on a logarithmic scale. The dotted line was determined by linear regression; the correlation coefficient (R² value) was 0.997.

**Effects of Longer Chain Alcohols (Hexanol and Heptanol).** We were unable to get consistent results with n-alcohols with chain lengths longer than pentanol, and upon careful examination, it seemed as though, with alcohols of carbon chain length of 6 or above, the solubility of the alcohol in our aCSF was incomplete. Despite trying numerous techniques for increasing solubility, we were unable to maintain these alcohols in solution consistently to perform the detailed concentration-response analysis. Therefore, the present study limited itself to n-alcohols of pentanol and shorter chain length.

**Effect of Trichloroethanol.** Trichloroethanol (TCE) is the active metabolite of chloral hydrate (Hobbs et al., 1996), and we assessed its effect on DA VTA neuronal firing. TCE produced a concentration-dependent excitation of DA VTA neurons over the concentration range from 0.5 to 2.5 mM (n = 7) (Fig. 5). Higher concentrations produced apparent depolarization block of firing (data not shown), as noted above for the n-alcohols, and so these data were not included in the concentration-response curve. Fitting a sigmoidal curve to the data and calculating the EC₄₀ yields a value of 1.28 mM. This indicates that TCE excites DA VTA neurons approximately 10 times more potently than ethanol itself.

**Effect of Ethanol and Butanol on Firing Frequency and Membrane Potential in Dissociated DA VTA Neurons.** Finally, we examined the effect of high concentration of ethanol and butanol on dissociated DA VTA neurons using nystatin-perforated patch current-clamp recording. We have previously reported that dissociated DA VTA neurons are excited by ethanol similarly to DA VTA neurons in brain slice preparations (Brodie et al., 1999b). All of the VTA neurons examined in the present study were identified as DA neurons based on the electrophysiological criteria as described previously (Koyama and Appel, 2006). Figure 6A shows the typical response of a DA VTA neuron to 360 mM ethanol. The high concentration of ethanol depolarized membrane potential, decreased action potential (AP) amplitude, and ceased AP generation (Fig. 6A). Before the treatment with ethanol, we assessed the resting membrane potential, which was determined as the membrane potential measured at 60 ms before the peak of the spontaneous AP (Brodie et al., 1999a). During the treatment with ethanol, we measured the membrane potential in which spontaneous AP generation was ceased. In four DA VTA neurons, 300 to 360 mM ethanol significantly depolarized membrane potential (−43.0 ± 0.7 mV in control, −39.0 ± 0.7 mV with ethanol; p < 0.01) with a significant decrease in firing frequency (0.9 ± 0.1 Hz in control, 0.2 ± 0.1 Hz with ethanol; p < 0.01). Figure 6B shows the typical response of a DA VTA neuron to 40 mM butanol. The high concentration of butanol depolarized the membrane potential and decreased AP amplitude and firing frequency (Fig. 6B).

In the similar manner described above, we measured membrane potentials before and after the treatment with butanol. In four DA VTA neurons, 40 mM butanol significantly depolarized membrane potential (−45 ± 0.3 mV in control, −42 ± 0.8 mV with butanol; p < 0.05) with a significant decrease in firing frequency (1.0 ± 0.3 Hz in control, 0.2 ± 0.1 Hz with butanol; p < 0.05).

**Discussion**

We have previously shown that ethanol increases the firing rate of DA VTA neurons in brain slices and of acutely dissociated DA VTA neurons. In the present report, we present evidence that n-alcohols of carbon chain length from 1 to 5 increased the firing frequency of DA VTA neurons. For each alcohol, the increase in firing frequency was concentration-dependent and reversed with washout of the alcohol. This indicates that like many other ethanol-related phenomena, the ethanol-induced increase in firing of DA VTA neurons is not a phenomenon exclusive to ethanol but is true for other n-alcohols. Although the apparent effects of each alcohol is similar, we do not know whether each alcohol acts at the same sites to produce the increase in firing and the apparent depolarization block of spontaneous firing. Substantial additional experiments would be required to determine whether ethanol and the other n-alkanols act at the same site(s) on DA VTA neurons, or whether each alcohol has a different spectrum of sites at which it acts. Early studies also showed a relation-
ship between chain length and the behavioral effects of \(n\)-alkanols (Wallgren, 1960; McCrery and Hunt, 1978; Lyon et al., 1981). The rate of increase in potency with increasing chain length reported in the literature differs in each study, with an increase in potency of approximately 2 to 4 times per additional carbon group (McCreery and Hunt, 1978; Lyon et al., 1981), which is in agreement with our data suggesting that each additional carbon produces an approximately 3- to 4-fold reduction in the concentration of ethanol, which is needed to produce a 40\% increase in firing rate (EC\(_{40}\)) (Fig. 4A) or apparent depolarization block (Fig. 4B). One caveat is that different degrees of volatilization of the different alcohols may slightly change the bath concentrations from the calculated concentration. Because excitation of the VTA has been related to the rewarding and reinforcing properties of drugs of abuse (Wise, 1996), this would suggest that other alcohols might also be subject to abuse.

In addition to the action of other \(n\)-alcohols on the firing rate of DA VTA neurons, we showed that TCE, which is the active metabolite of chloral hydrate, increases the firing frequency of DA VTA neurons. Like the \(n\)-alcohols, this action of TCE could underlie the reinforcing action of chloral hydrate (Robinson, 1966). Other groups have also observed ethanol-like actions of TCE in other preparations (Krasowski et al., 1998; Lovinger et al., 2000). One point of note is that the level of excitation of DA VTA neurons produced by TCE without apparent depolarization block was higher than those observed with ethanol. That is, TCE was capable of increasing the firing rate up to 120\% of baseline, whereas ethanol excitation rarely exceeded a 50\% increase (Fig. 2). Additional studies, using finer gradations of alcohol concentrations, will be necessary to firmly establish this point, but if confirmed, this could indicate that there are subtle differences in the selectivity or efficacy of different alcohols for the spectrum of sites on DA VTA neurons responsible for their excitation. This information could be important for the development of agents to interfere with the reinforcing/rewarding properties of ethanol.

Depolarization blockade is a known phenomenon of neurons in general and dopamine neurons in particular. The therapeutic action of antipsychotics has been related to the development of chronic depolarization blockade over time (Grace and Bunney, 1986; Deutch et al., 1991). In the present study, we have shown that high concentrations of \(n\)-alcohols can produce acute and reversible apparent depolarization block. We also observed that high concentrations of TCE can produce apparent depolarization block. Because we cannot record membrane potential with the extracellular recording methods we used in the majority of the experiments reported here, we cannot be certain that the cessation of firing was because of depolarization of the membrane. To assess whether the block of firing observed with high concentrations of \(n\)-alkanols might be associated with depolarization, we tested acutely dissociated DA VTA neurons with high concentrations of ethanol and butanol. The cessation of firing of
these neurons was associated with a depolarization of the membrane. Additional experiments will be necessary to ascertain whether the magnitude of depolarization alone is enough to account for the cessation of firing, or whether other effects of ethanol on ion channels in the membrane, in addition to the depolarization, are necessary for the blockade of firing produced by high alcohol concentrations. Although the apparent depolarization blockade phenomenon makes it difficult to develop complete concentration-response curves, it indicates that the depolarization of the neuronal membrane by ethanol and other alcohols is a concentration-dependent effect (Brodie and Appel, 1998). The concentrations at which apparent depolarization blockade of firing is produced by ethanol greatly exceed the lethal concentration, so in general, it is unlikely the apparent depolarization blockade plays a role in the acute effects of ethanol in alcohol-naive animals. It has been reported that withdrawal from chronic ethanol can produce a state of depolarization blockade in DA VTA neurons (Shen and Chioido, 1993).

There were linear relationships between excitatory effects of n-alcohols and their lipophilicity, and between the concentration at which apparent depolarization blockade occurred and lipophilicity. This does not necessarily indicate that the site of action of the alcohols is at the lipid of the membrane because others have observed the same relationship for a clearly defined ion channel protein target (Covarrubias et al., 1995). Finding a cutoff point, a carbon chain length at which n-alcohols would have no excitatory effect, was our initial goal, but that goal was thwarted by solubility problems. It was encouraging, however, that TCE had an excitatory effect on DA VTA neurons; this indicates that chemical modifications of the ethanol molecule can be made that may increase our understanding of the alcohol binding pocket on specific ion channels in DA VTA neurons. Additional experiments isolating alcohol-sensitive proteins will be necessary to determine whether there is indeed a protein target of alcohol action in DA VTA neurons. We have shown that ethanol excitation can be blocked by quinidine (a blocker of some delayed rectifier potassium channels) (Appel et al., 2003), which suggests that ethanol is acting on an ion channel in the membrane of DA VTA neurons.

These studies have shown that, like ethanol, n-alcohols and TCE can increase the firing frequency of DA VTA neurons and can excite to the point of apparent depolarization blockade. The relationship between lipophilicity and magnitude of the action of the alcohols on DA VTA neurons indicates that there is a hydrophobic site on the target ion channel in these neurons that is the site of action of ethanol. Ongoing work in our laboratories will identify the specific ion channels in DA VTA neurons that mediate the ethanol excitation. Additional studies will be needed to determine the exact location of the hydrophobic pocket that represents the site of alcohol action.

References


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