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Aliphatic alcohols increase the decay rate of glutamate-activated currents at the crayfish neuromuscular junction

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1 Excitatory junction currents produced by glutamate were recorded with an extracellular electrode at the excitatory neuromuscular junction of the crayfish.

2 The currents decayed more quickly as the membrane was hyperpolarized. The direction of the voltage sensitivity of the decay phase is thus opposite to that found for acetylcholine-activated currents at the amphibian endplate.

3 The aliphatic alcohols ethanol to octanol all increased the rate of decay of the currents. The effects of the short chain alcohols were opposite to their actions at the toad endplate, where ethanol to pentanol prolong the currents. This observation was explained in terms of the opposite direction of the voltage sensitivity in the two preparations.

4 For each alcohol, the relationship between the half-decay time of the currents ($t_{1/2}$) and alcohol concentration was exponential.

5 The potency of each alcohol in decreasing $t_{1/2}$ was exponentially related to carbon chain length, which would be predicted if the effects of the alcohols were directly related to their concentration in the lipid phase of the membrane.

6 These findings are consistent with the ideas that the alcohols may alter membrane polarizability or change membrane fluidity in the vicinity of the channels.

Introduction

At the neuromuscular junction, the rate of decay of postsynaptic currents is often a function of membrane potential. In amphibia, miniature endplate currents (m.e.p.cs) produced by acetylcholine decay more slowly as the membrane is hyperpolarized. At the crayfish neuromuscular junction, however, the decay phase of excitatory junction currents (e.j.cs) produced by glutamate is faster at more hyperpolarized membrane potentials (Dudel, 1977; Onodera & Takeuchi, 1978). The voltage sensitivity of the decay rate of glutamate-activated currents in the crayfish is thus opposite to that found for acetylcholine-activated currents at the toad endplate.

The decay of postsynaptic currents is thought to depend on the rate at which transmitter-activated channels relax back to their closed state. Channel closure may be associated with the movement of a dipole within the membrane, and the rate at which channels close may therefore be influenced by the electric field across the membrane (Magleby & Stevens, 1972). Presumably, these dipoles are oriented in opposite directions in amphibia and crayfish, since hyperpolarization has opposite effects on the decay of postsynaptic currents in the two preparations.

The aliphatic alcohols ethanol to octanol have been shown to alter the decay phase of m.e.p.cs in the toad, and it has been suggested that they may be affecting the environment of the dipole associated with channel gating. The short chain alcohols ethanol to pentanol prolong m.e.p.c. decay in the toad, perhaps by altering the polarizability of the membrane in the vicinity of the channels (Gage *et al.*, 1975). M.e.p.cs are shortened by octanol, which may act by increasing the fluidity of membrane lipids near the channels (Gage *et al.*, 1974; 1978). The alcohols appear to act by partitioning into the lipid phase of the membrane and altering the local environment of the acetylcholine receptor-channel complexes.

In order to test these ideas regarding the alcohols,

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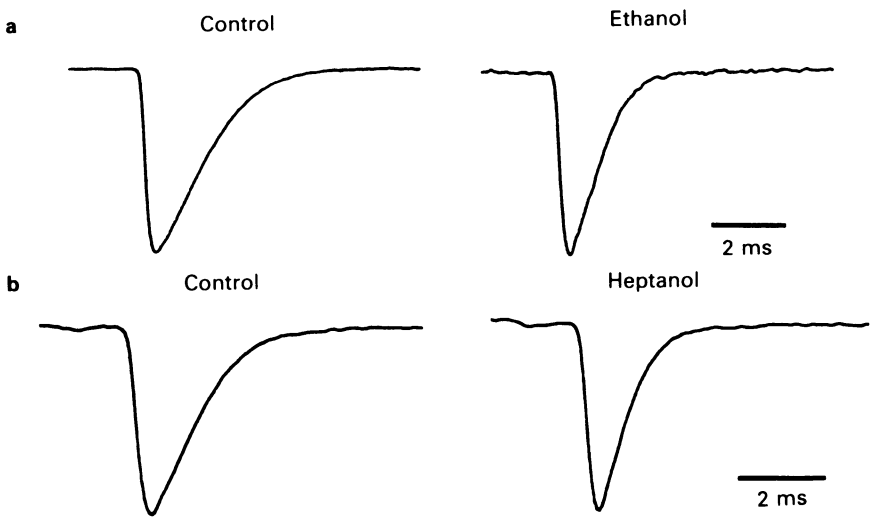

Figure 1 Ethanol and heptanol increase the decay rate of excitatory junction currents. (a) Averaged e.j.cs obtained in the absence and presence of ethanol. Ethanol 100 mM decreased $t_{1/2}$ by 36%, from 1.85 to 1.18 ms, and A_I by 37% from 2.13 to 1.35. (b) Averaged e.j.cs obtained in the absence and presence of 1 mM heptanol. In this experiment, $t_{1/2}$ decreased by 38%, from 1.18 to 0.73 ms, while A_I decreased by 33%, from 1.32 to 0.89. Each record is the average of 61–127 currents. The deflections at the beginning of the traces are extracellular action potentials, which were broadened during averaging of the e.j.cs. Currents have been normalized to the same peak height.

their effects on the decay phase of e.j.cs produced by glutamate were studied at the excitatory neuromuscular junction of the crayfish. If the short chain alcohols do indeed act by altering membrane polarizability, then they should have opposite effects in toads and in crayfish due to the opposite orientations of the dipoles in the two preparations. Ethanol through pentanol should increase the decay rate of e.j.cs, as shown for ethanol in the crab (Adams *et al.*, 1977). Octanol, if it increases membrane fluidity, should have similar effects in both toads and crayfish, and should shorten the decay phase of e.j.cs.

An analysis of the decay rate of e.j.cs in the crayfish demonstrates that the effects of the aliphatic alcohols are consistent with these predictions. Ethanol through octanol shorten the decay phase of e.j.cs and appear to act via the lipid phase of the membrane. The effects of the alcohols are compatible with the idea that they alter membrane polarizability or increase membrane fluidity in the vicinity of the channels.

Methods

The methods used in these experiments have been described previously (Wachtel, 1984). Excitatory junction currents (e.j.cs) produced by nerve stimulation were recorded with an extracellular electrode from junctional regions of muscle fibres in the abductor of the dactyl of crayfish (*Cherax destructor*) walking legs.

The nerve innervating the leg was dissected free in the meropodite, and the excitatory and inhibitory axons innervating the abductor were separated from each other. The excitatory axon was then stimulated with a suction electrode, usually at 4–8 Hz. The stimulation rate was selected to produce a high percentage of failures, so that a maximum of 1 or 2 quanta of transmitter were usually released in response to each stimulus.

To determine the voltage sensitivity of current decay, membrane potential was altered by means of a 2 electrode voltage clamp. E.j.cs were still recorded extracellularly and the electrodes were shielded to minimize capacitive coupling between the extracellular electrode and the current-passing electrode.

Currents were aligned at their peaks before averaging. The decay phase of averaged e.j.cs was described by a $t_{1/2}$, or the time required to decay to one-half the peak height, and also by A_I, the area under a normalized peak. The area A_I was calculated by summing the digitized points of the averaged e.j.c., then dividing by the peak height. Thus A_I is in units of time (ms). In order to compare data from different experiments, values of $t_{1/2}$ and A_I obtained after addition of the alcohols were often expressed as a percentage of control values.

All alcohols were reagent grade. Concanavalin A was purchased from Sigma and Calbiochem, and

solutions (0.25–5 μM) were always prepared immediately before use. Experiments were performed at 11–14 °C.

Results

Alcohols shorten current decay

Figure 1 illustrates the effects of ethanol and heptanol on averaged e.j.cs. The currents decayed more rapidly after addition of both alcohols, and $t_{1/2}$ and A_I were decreased.

All of the alcohols tested, ethanol through octanol, shortened the decay phase of e.j.cs. The basic shape of the decay was not systematically altered by any of the alcohols, and the ratio between $t_{1/2}$ and A_I never changed by more than about 10%. For the 7 alcohols combined, the ratio $t_{1/2}$:A_I changed from 0.852 ± 0.012 to 0.857 ± 0.017 (mean ± s.e.mean) which is not significant ($P > 0.8$; Student's paired t test).

As the concentration of alcohol was increased, the e.j.cs decayed faster. The relationship between alcohol concentration and the decrease in $t_{1/2}$ is shown in Figure 2 for ethanol and heptanol. At low concentrations of each alcohol, $t_{1/2}$ decreased exponentially with concentration. At 2 mM heptanol, though, the observed value for $t_{1/2}$ was less than expected. The measured value of $t_{1/2}$ was 0.6 ms, while extrapolation of the line fit from 0 to 1 mM would predict that $t_{1/2}$ should be 0.4 ms. However, values of $t_{1/2}$ less than 0.5 ms were not usually observed, and it is possible that the decay rate of the e.j.cs reached some limiting value. This trend was observed with all the alcohols when $t_{\frac{1}{2}}$ approached 0.5 ms.

At decay rates where $t_{1/2}$ was greater than 0.5 ms, the relationship between $t_{1/2}$ and concentration was exponential, according to the equation $t_{1/2} = t^0_{1/2} \exp(-B_N \times C_N)$, where $t^0_{1/2}$ is the half-decay time in the absence of alcohol, C is the concentration of alcohol of carbon chain length N, and B_N is a constant, different for each alcohol (Gage *et al.*, 1975).

Values of B_N for each alcohol were calculated from the slope of dose-response curves, similar to those shown in Figure 2. The concentration of alcohol needed to produce a given reduction in $t_{1/2}$ decreased dramatically with increasing chain length, and the relationship between B_N and carbon chain length N was exponential (Figure 3). Since the relationship between membrane/buffer partition coefficients and N is also exponential (Roth & Seeman, 1972), this suggests that the effectiveness of an alcohol may be directly related to its concentration in the lipid phase of the membrane (Gage *et al.*, 1975).

This result is analogous to the relationship between B_N and N at the toad endplate, where B_N is also exponentially related to N. However, the effects of the lower chain alcohols in the crayfish are opposite to their effects at the toad endplate, where ethanol through pentanol prolong m.e.p.c. decay. This is consistent with the opposite direction of the voltage sensitivity of current decay in the two preparations,

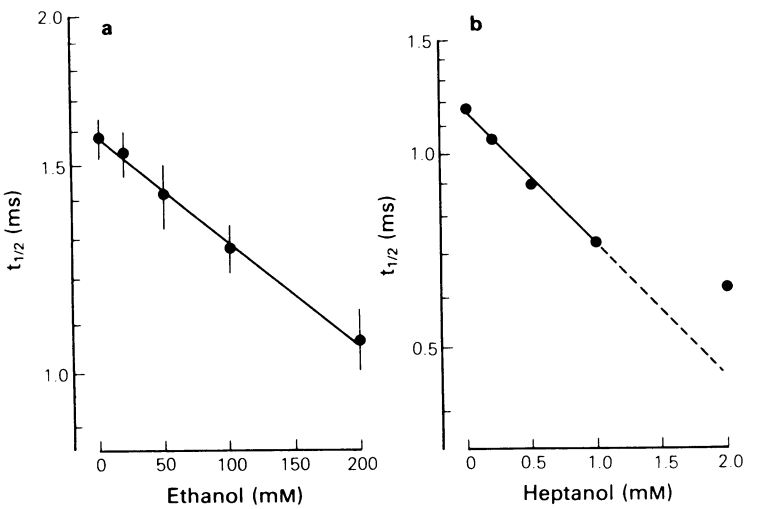

Figure 2 $t_{1/2}$ as a function of alcohol concentration for ethanol and heptanol. Note logarithmic ordinates. (a) Ethanol curve represents pooled data from 10 experiments. Vertical lines represent ± 1 s.e.mean. Line is a least squares fit to the points 0–200 mM. (b) Heptanol curve shows the results of a single experiment. Line is a least squares fit from 0.1–1.0 mM.

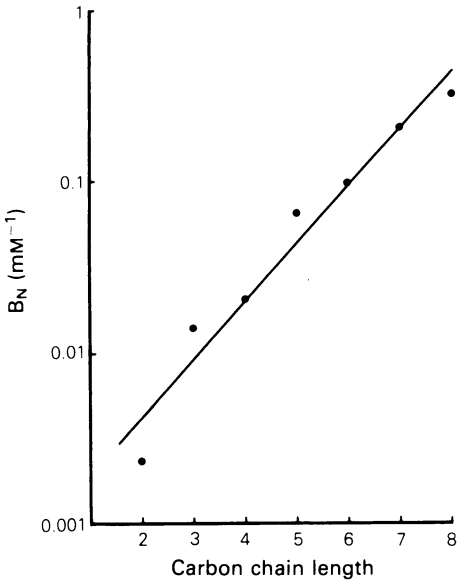

Figure 3. B_N (see text, Results) as a function of carbon chain length N for ethanol through octanol. B_N for each alcohol was calculated from the slope of dose-response curves, as shown in Figure 2. The solid line is a least squares fit. The point for ethanol was calculated from the results of 10 experiments, while data for each of the other alcohols is from 2–5 experiments.

assuming that alcohol effects are indeed related to the orientation of the dipole associated with channel gating.

Ethanol does not alter voltage sensitivity

In a few experiments, e.j.cs were recorded extracellularly while the membrane potential of the fibre was shifted using a standard 2-electrode voltage clamp. Hyperpolarization caused the currents to decay faster, confirming previous reports on the voltage sensitivity of the decay (Dudel, 1977; Onodera & Takeuchi, 1978).

The relationship between $t_{1/2}$ and membrane potential was linear over the range tested, −60 to −140 mV (Figure 4). Ethanol caused the currents to decay faster at all membrane potentials, although the voltage sensitivity of $t_{1/2}$ was slightly reduced as the ethanol concentration was increased. For the fibre of Figure 4, a change in membrane potential of 100 mV in the absence of ethanol produced a change in $t_{1/2}$ of 0.48 ms, whereas in the presence of 100 mM ethanol $t_{1/2}$ changed by 0.39 ms 100 mV^{-1}, and in 200 mM ethanol $t_{1/2}$ changed by 0.32 ms 100 mV^{-1}.

Ethanol and concanavalin A

Concanavalin A (con A) is a plant lectin which has been reported to eliminate desensitization and abolish the voltage sensitivity of current decay at glutamate synapses in the crayfish (Dudel, 1979; Shinozaki & Ishida, 1979; Stettmeier *et al.*, 1983). In order to remove the voltage sensitivity of current decay, con A presumably eliminates the change in dipole movement which occurs when a channel opens or closes, or at least suppresses the influence of the membrane field on the change in dipole movement. If the effects of ethanol were dependent on this change in dipole movement, then ethanol should not be effective when applied together with con A.

Unfortunately, con A was relatively ineffective in abolishing the voltage sensitivity of $t_{1/2}$ in this preparation. In 7 fibres exposed to 0.25–5 μM con A for 40–60 min, the average voltage sensitivity was 0.58 ± 0.16 ms 100 mV^{-1}, compared with 0.48 ± 0.03 in the absence of con A.

In addition, con A was not able to prevent ethanol from increasing the rate of current decay, regardless of which drug was applied first. In three fibres exposed to 200 mM ethanol plus con A at the resting potential, $t_{1/2}$ was 1.10 ± 0.12 ms (mean ± s.e.mean), compared with 1.06 ± 0.07 for 6 fibres exposed to 200 mM ethanol alone. This finding is rather inconclusive however, due to the ineffectiveness of con A when applied alone.

Discussion

At the toad endplate, the alcohols have varying effects on the decay of m.e.p.cs. Ethanol to pentanol

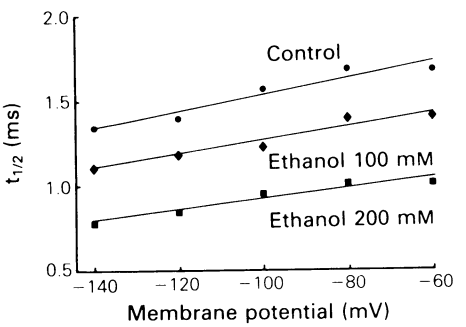

Figure 4 Effect of ethanol on the voltage dependence of excitatory junction current (e.j.c.) decay. $t_{1/2}$ is plotted as a function of membrane potential in the absence and presence of ethanol. E.j.cs were recorded extracellularly while membrane potential was altered with a 2-electrode voltage clamp. The slopes of the lines are 0.48 ms 100 mV^{-1} in control, 0.39 ms 100 mV^{-1} in 100 mM ethanol and 0.32 ms 100 mV^{-1} in 200 mM ethanol. All data are from a single junctional region.

lengthen m.e.p.cs., hexanol sometimes causes the decay to appear biphasic, while octanol shortens m.e.p.c. decay. The shorter chain alcohols may produce local alterations in the polarizability of the membrane, thereby increasing the stability of the channel in its open state (Gage *et al.*, 1975). Octanol appears to increase membrane fluidity near the channels, and enhances the rate at which open channels close (Gage *et al.*, 1978). All of the alcohols however, are thought to act in the lipid phase of the membrane.

The results of the experiments at the excitatory neuromuscular junction of the crayfish, where all of the alcohols shorten the decay phase of e.j.cs, are consistent with these ideas. The alcohols appear to act via membrane lipids, or hydrophobic regions of the membrane, since their effects are directly related to their concentration in the lipid phase of the membrane. The relationship between carbon chain length and alcohol potency, determined from the slope of dose-response curves, is exponential. Membrane/buffer partition coefficients are also exponentially related to carbon chain length (Roth & Seeman, 1972), implying that the potency of an alcohol is directly related to its partition coefficient.

The alcohols could increase the decay rate of e.j.cs either by altering membrane polarizability or by increasing membrane fluidity. However, since both changes in membrane polarizability and increases in membrane fluidity would be expected to shorten e.j.c decay, it is not possible to separate these two effects.

If con A had been effective in abolishing the voltage sensitivity of e.j.c. decay, it would have been useful for determining the extent to which membrane polarizability was related to the effects of the alcohols. Unfortunately, con A purchased from two different suppliers was not able to eliminate the effects of voltage on the decay of e.j.cs.

However, experiments by Magazanik & Vyskocil (1979) at excitatory synapses of fly larval muscles argue against the idea that the effects of ethanol may be related to changes in membrane polarizability. In this preparation, where glutamate is an agonist, ethanol also shortens the decay of postjunctional currents. However, hyperpolarization lengthens current decay, and thus the voltage-dependence resembles that found at the toad endplate, and not the crayfish. This result is in direct contradiction to the idea that the effects of ethanol are mediated by changes in membrane polarizability.

Although it is not certain whether the alcohols act by causing changes in membrane polarizability or membrane fluidity, the evidence is strong that these compounds do act in the lipid phase of the membrane. Once they have partitioned into the membrane, the alcohols must somehow modify the local environment of the receptor-channel complexes, and thus alter the stability of the open state.

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