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Ethanol as a general anesthetic: actions in spinal cord

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Abstract

Ethanol, usually studied in relation to intoxication, is also capable of producing general anesthesia. The most common standard of anesthetic potency is the concentration which produces immobility in response to a noxious stimulus. This concentration will be referred to as the anesthetic concentration. Immobilization is a spinal effect. Ethanol effects were studied in spinal cord from 2–7-day-old rats at concentrations which included the anesthetic concentration in both adult rats (97 mM) and 6–7-day-old rats (235 mM). At neonatal but not adult anesthetic concentrations, ethanol depressed monosynaptic reflex amplitude (mediated by glutamate AMPA receptors + compound action potential). At both neonatal and adult anesthetic concentrations ethanol reversibly depressed the population excitatory postsynaptic potential (pEPSP) (glutamate AMPA and NMDA receptors), the slow ventral root potential (NMDA + metabotropic receptors), and the dorsal root potential ($GABA_A$ receptors, via glutamate-excited interneurons). Effects were greater on NMDA receptor-mediated components than on AMPA-receptor-mediated components of the pEPSP and greater on NMDA than on metabotropic receptor-mediated components of the slow ventral root potential. The profile of ethanol effects on spinal cord resembles that of inhalation general anesthetics. The results show that both AMPA and NMDA receptor-mediated transmission are sensitive to ethanol and that enhancement of GABAergic neurotransmission is overridden by depression of excitation to the interneurons. They provide no obvious explanation for ethanol's lower general anesthetic potency in the neonate. © 1997 Elsevier Science B.V.

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1. Introduction

No drug has had a longer history of human use than ethanol (ethyl alcohol). Although primarily of social interest and concern as an intoxicant and drug of abuse, ethanol can produce a state of general anesthesia and historically has been used for this purpose (Dundee et al., 1969). Ethanol causes amnesia and loss of consciousness in man, and in experimental animals can cause immobility in response to a noxious stimulus. Immobility in response to a noxious stimulus is the endpoint of the most common operational definition of general anesthesia. For inhaled agents, this is the minimal alveolar concentration at which 50% of a population fails to move in response to surgical incision or tail clamp (Quasha et al., 1980). This will be referred to as the anesthetic concentration.

For inhalation agents in clinical use, the anesthetic concentration correlates very well with the oil/gas partition coefficient. The long-standing Meyer-Overton correlation between anesthetic potency and lipid solubility (Meyer, 1899; Overton, 1901), holds for a wide variety of compounds of many different structures. For these compounds the product of the anesthetic concentration × oil/gas partition coefficient is roughly constant, with a value approximately 2. For a series of clinical anesthetic agents, the product of the anesthetic concentration determined in rats and the olive oil/gas partition coefficient is 1.82 ± 0.56 (mean ± S.D.) (Taheri et al., 1991). However, a number of compounds have recently been characterized that violate the Meyer-Overton correlation (Koblin et al., 1994). These compounds, highly halogenated structures, have lipid solubilities that predict they should be anesthetics. Some have anesthetic concentration × oil/gas partition coefficient products much greater than 2. Others appear to have no potency to immobilize animals at all, in spite of oil/gas partition coefficients within the anesthetic range. The properties of these agents give rise to the question, what do they not do that is a determining factor in the production of general anesthesia? Short straight-chain alcohols, on the other hand, violate the Meyer-Overton correlation in the opposite direction: in adult rats they are more potent than

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their lipid solubilities predict, with an olive oil/gas × anesthetic concentration product of 0.1–0.2 (Fang et al., unpublished data). This constant is an order of magnitude less than the constant derived from the Meyer-Overton correlation. The properties of alcohols give rise to the question, what are they doing that makes them more potent agents than predicted?

For inhalation agents, the immobility associated with the anesthetic concentration is a function of anesthetic actions in the spinal cord (Antognini and Schwartz, 1993; Borges and Antognini, 1994; Rampil, 1993, 1994; Rampil et al., 1993). We have examined the actions of ethyl alcohol in an isolated spinal cord preparation from rats 2–7 days old. This preparation is intrinsically relevant to the abolition of movement produced by anesthetic agents, and we have used it in a number of previous studies to profile the actions of representatives of several classes of general anesthetic agents (Brockmeyer and Kendig, 1995; Collins et al., 1995; Feng and Kendig, 1995a; Jewett et al., 1992; Kendig et al., 1991; Savola et al., 1991). With respect to ethanol, the preparation provides access to several pathways employing transmitter receptors that have been implicated in ethanol's intoxicating actions as well as in theories about the mechanisms of general anesthesia. These are glutamate NMDA and non-NMDA receptors, and $GABA_A$ receptors. The present study examined the actions of ethyl alcohol on these and other receptor-mediated pathways in spinal cord.

In separate parallel studies, anesthetic concentrations were determined in rats of the same age of 6–7 days. Ethanol anesthetic concentrations were found to be very dependent on age (Fang et al., 1997). Possible reasons for the age dependence of anesthetic potency are addressed in Section 4.

2. Materials and methods

Spinal cords from 2–7-day-old rats were removed following decapitation under enflurane anesthesia in a protocol approved by Stanford's animal care and use committee. Isolated spinal cords were perfused at 4 ml/min with artificial cerebrospinal fluid (ACSF) at 27–28°C, equilibrated with 95% O_2/5% CO_2, pH 7.3–7.4. The temperature is the one measured by a rectal probe in rats of this age when not under the mother. The pre-equilibrated ACSF was delivered from glass syringes mounted on an infusion pump set to a constant rate of approximately 4 ml/min. ACSF was of the following composition (mM): NaCl 123, KCl 5, NaH_2PO_4 1.2, $MgSO_4$ 1.3, $NaHCO_3$ 26, $CaCl_2$ 2, glucose 30.

To evoke and record population evoked potentials from the spinal cord, suction electrodes were arranged to stimulate a lumbar dorsal root and record either from the corresponding ipsilateral ventral root (monosynaptic reflex and slow ventral root potential), from an ipsilateral ventral root offset 1–2 segments from the dorsal root being stimulated (population excitatory postsynaptic potential, EPSP) or from an adjacent dorsal root (dorsal root potential). Single stimuli 0.2 ms in duration, 9 V nominal intensity were delivered to the dorsal root at a constant frequency of 1/50 s throughout the experiment. Responses were digitized, averaged in groups of 5, and stored for later analysis.

Ethanol was obtained from commercial sources, diluted to the desired concentration in ACSF and delivered to the preparation from syringes as outlined above. Concentrations of ethanol in the bath were verified by gas chromatography of the vapor phase in equilibrium with the solution in the chamber. Vapor phase partial pressures as per cent of one atmosphere V/V were converted into ACSF concentrations in mM by using a saline-gas partition coefficient of 2650 at 37°C to give the equivalent saline partial pressure in ml/liter, correcting for the difference from absolute temperature and dividing by Avogadro's number.

Four to twelve preparations were exposed to a single concentration of ethanol in each type of experiment. The following measurements were made: monosynaptic reflex amplitude; population excitatory postsynaptic potential area; the area under the curve of the first 8 s of the slow ventral root potential; dorsal root potential area. In some experiments on the population excitatory postsynaptic potential, the selective glutamate receptor antagonists (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphoric acid (CPP) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were used to block NMDA and AMPA receptor-mediated components respectively. The slow ventral root potential was divided for analysis into two components, a relatively fast component sensitive to NMDA receptor antagonists 80–440 ms after the stimulus, and a slower component sensitive to a variety of metabotropic receptor antagonists 2.8–7.8 s after the stimulus. Data on amplitude and area were normalized to individual control values and analyzed by *t*-test.

3. Results

Fig. 1 shows examples of the effects of ethanol on each of the spinal cord evoked potentials examined in this study: the monosynaptic reflex, the population excitatory postsynaptic potential, the slow ventral root potential and the dorsal root potential. In each case the response was depressed; the depressant effects were reversible on washing with ethanol-free ACSF.

Ethanol dose-dependently decreased the amplitude of the monosynaptic reflex (Fig. 2A). The depression was significant at the anesthetic concentration for neonatal but not for adult rats (indicated by dotted and solid lines respectively). These concentrations were determined in separate studies (Fang et al., 1997). The monosynaptic reflex is the compound action potential of motor neurons

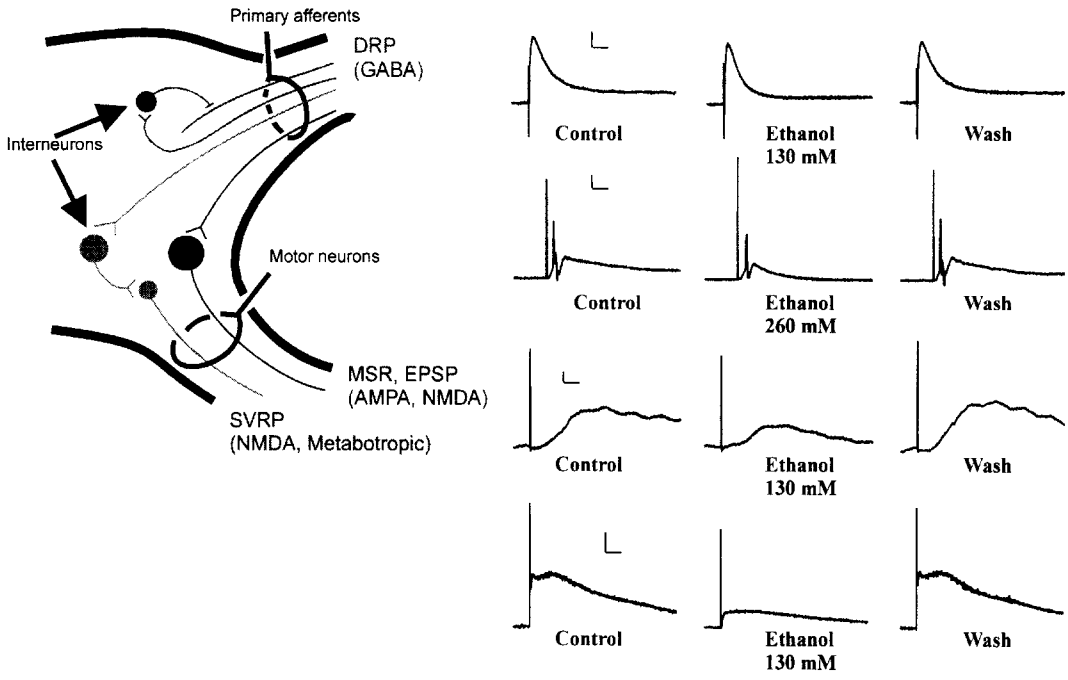

Fig. 1. Diagram of the circuitry in the spinal cord and examples of the actions of ethanol on evoked potentials. The dorsal root potential (DRP) is a presynaptic depolarizing inhibitory response to GABA released from interneurons and acting on $GABA_A$ receptors on primary afferent nerve terminals. The GABAergic interneurons are excited by glutamate acting on both NMDA and non-NMDA receptors. The monosynaptic reflex (MSR) and its underlying excitatory postsynaptic potential (EPSP) are mediated by glutamate released from primary afferents acting on glutamate AMPA receptors on the motor neurons, with some component of NMDA receptor activity. The slow ventral root potential (SVRP) is a complex depolarizing response with an early glutamate NMDA receptor-mediated component and a late component mediated by several metabotropic receptors including those for substance P. Examples of ethanol effects on these responses are shown at the right from top to bottom: dorsal root potential, monosynaptic reflex, EPSP, slow ventral root potential. The associated calibration marks are respectively for amplitude 0.2 mV, 1 mV, 0.5 mV and 0.1 mV; for time scale 200 ms, 10 ms, 10 ms and 2 s.

in the ventral root, triggered when glutamate released from primary afferent nerve terminals generates a depolarizing excitatory postsynaptic potential which exceeds the threshold at the spike initiating zone. Changes in the monosynaptic reflex thus may reflect changes in either synaptic transmission or impulse initiation, or both. The population EPSP recorded from ventral roots 1–2 segments away from the stimulating electrode on the dorsal root reflects the spread of primary afferent terminals to heterosegmental motor neurons, but with a synaptic strength insufficient to trigger an action potential. It thus provides a convenient way to record the monosynaptic EPSP uncontaminated by the compound action potential of the monosynaptic reflex (Tauck and Kendig, 1996). Fig. 1 shows an example of the EPSP and the effects of ethanol, and Fig. 2B shows the effects of ethanol on the area of the population EPSP. The response was depressed, suggesting that depression of synaptic transmission contributes to ethanol's effects on

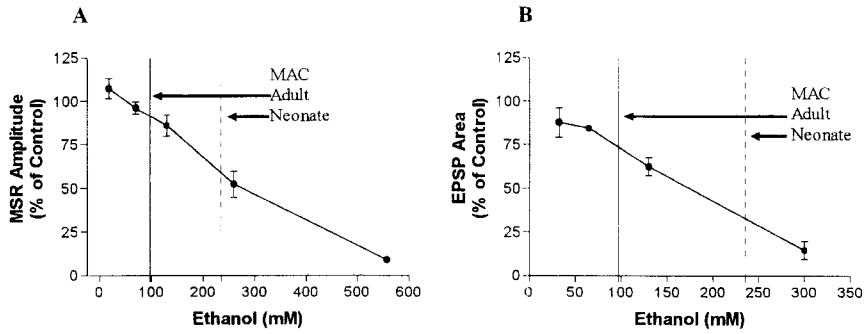

Fig. 2. (A) Ethanol depresses the amplitude of the monosynaptic reflex (MSR). The depression is significant ($P < 0.05$) at the anesthetic concentration (MAC) for neonatal but not adult rats (indicated by solid and dotted lines, respectively). (B) Ethanol also depresses the population EPSP observed when stimulating and recording electrodes are offset 1–2 segments from each other. EPSP depression is significant ($P < 0.05$) at both adult and neonatal anesthetic concentrations (MAC). Data points are means of 4–6 individual experiments, each spinal cord exposed to a single concentration of ethanol. Error bars are S.E.M.

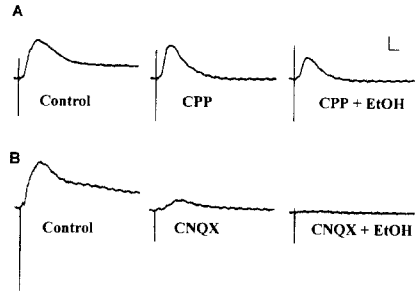

Fig. 3. Ethanol depresses both the AMPA and NMDA receptor-mediated components of the population EPSP evoked by stimulating a lumbar dorsal root and recording from a ventral root 1–2 segments away. (A) The competitive NMDA receptor antagonist CPP (5 μM) removes slow and very slow components of the EPSP, leaving a fast-rising and decaying depolarization sensitive to AMPA receptor antagonists. (B) The glutamate AMPA-kainate receptor antagonist CNQX (5 μM) removes the fast component and very slow components, leaving a slow-rising and decaying depolarization sensitive to NMDA receptor antagonists. Ethanol (EtOH) (130 mM) reduces the amplitude of both. Calibration marks are 1 mV and 10 ms.

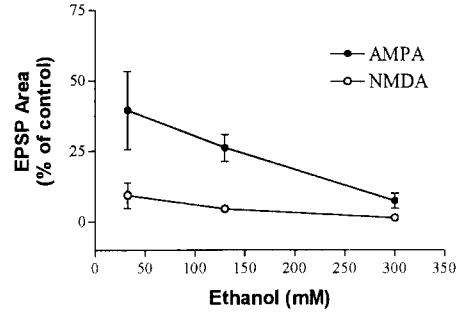

Fig. 4. AMPA and NMDA receptor-mediated components of the EPSP were isolated by treating with the antagonists CPP and CNQX respectively as shown in Fig. 3. Control values are those obtained after equilibration with the glutamate receptor antagonist. Ethanol depressed both at concentrations well below the adult or neonatal rat anesthetic (MAC) values. The NMDA receptor-mediated response was more sensitive to ethanol than the AMPA response. Data points are means of 4 experiments; error bars are S.E.M.

the monosynaptic reflex. Effects on the EPSP were significant at both adult and neonatal rat anesthetic concentrations.

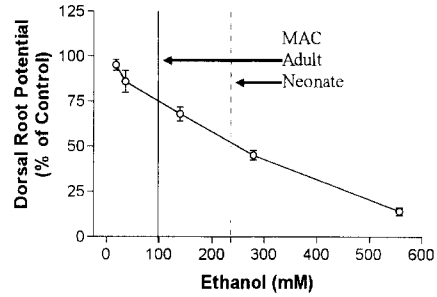

Fig. 6. Concentration-dependent depression of the dorsal root potential by ethanol. Data points are means of 4–6 individual experiments; error bars are S.E.M.

The population EPSP is composed of an early component sensitive to AMPA but not kainate or NMDA receptor antagonists, and a later smaller component sensitive to NMDA but not AMPA or kainate receptor antagonists (Tauck and Kendig, 1996). We examined the effects of ethanol on these components separately in experiments in which cords were treated with either CPP (an NMDA receptor antagonist) or CNQX (an AMPA/kainate receptor antagonist). Examples of the effects of ethanol on the isolated AMPA and NMDA receptor-mediated components of the population EPSP are shown in Fig. 3, and the comparative dose-response curves in Fig. 4. Ethanol depressed both AMPA and NMDA receptor-mediated responses, with the latter appearing to be more sensitive.

Fig. 5 shows the effects of ethanol on the slow ventral root potential in its entirety (Fig. 5A) and on the NMDA and metabotropic receptor-mediated components separately (Fig. 5B). The NMDA receptor component was slightly more sensitive than the later components, but the differences were not large.

Ethanol also depressed the dorsal root potential (Fig. 6).

4. Discussion

In the pattern of its effects on spinal cord evoked potentials ethanol closely resembles inhalation general anesthetics (Collins et al., 1995). In the present study, care

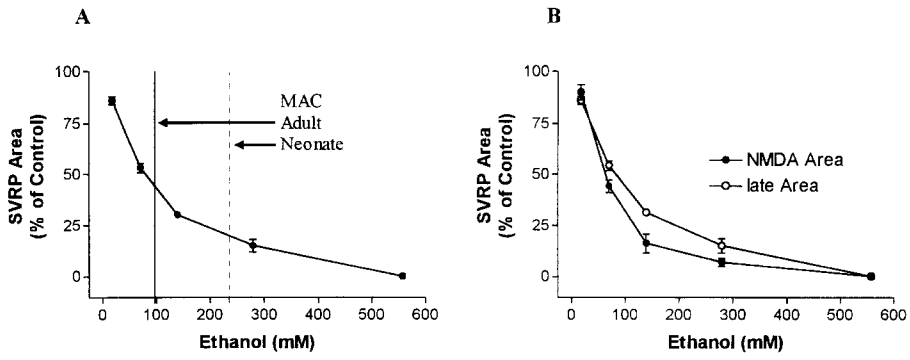

Fig. 5. (A) Ethanol depressed the area of the slow ventral root potential. (B) When the curve was analyzed separately for those early portions sensitive to NMDA receptor antagonists (NMDA area) and the remaining very slow component (late area) both were depressed; the NMDA component appeared slightly more sensitive. Data points are means of 4–7 individual experiments; error bars are S.E.M.

has been taken to examine the responses of the spinal cord to ethanol concentrations relevant to general anesthesia as defined by loss of movement in response to a noxious stimulus. In adult rats this concentration is 97 mM (Fang et al., 1997). Increases in general anesthetic potency with age are well known, the ratio of anesthetic concentrations in young to that in old ranging from 1.17 to 1.6 depending on age range and species (Fang et al., 1997). Age-related increases in ethanol sleep time, measured as time to regain righting reflex, have also been reported (Little et al., 1996). For ethanol, the difference in anesthetic concentration between 7-day-old and 3-month-old (adult) rats was much larger than for the inhalation agent desflurane, ethanol anesthetic concentration in young animals being 2.45 times that in older, whereas desflurane anesthetic concentration in young animals is only 1.19 times that in older (Fang et al., 1997). Ethanol thus appears to have a potency more influenced by age than inhalation general anesthetics.

In this study, we examined responses in the spinal cord of Sprague-Dawley rats from 2 to 7 days old. The spinal cord is the site at which anesthesia, as defined by lack of movement, is determined (Antognini and Schwartz, 1993; Borges and Antognini, 1994; Rampil, 1993, 1994; Rampil et al., 1993). The sensitivity of the cord to an anesthetic is thus appropriately related to the value required to abolish movement in that class of animal. Therefore the amount of ethanol required to abolish movement in the 7-day-old rat is the correct calibration point for an anesthetically relevant concentration. In the graphic representation of the data, both adult and 7-day-old rat anesthetic concentrations are indicated.

Intoxicating and analgesic (Bukusoglu et al., 1993) effects of ethanol are also of interest. Intoxication, like general anesthesia, is a complex state with multiple endpoints including amnesia and motor incoordination. Intoxicating concentrations of ethanol are reported in the range of 25–100 mM. In the present study, significant depressant effects of ethanol on the population EPSP, slow ventral root potential, and dorsal root potential were observed at concentrations of 65, 17.5, and 35 mM respectively; effects at these lower concentrations thus may also be relevant to the locomotor incoordination observed at intoxicating levels of ethanol. The slow ventral root potential is particularly relevant to nociception; depression of this response may contribute to the analgesic properties of ethanol.

In this study at the concentrations considered relevant to anesthesia but higher than those associated with intoxication, ethanol depressed the monosynaptic reflex. In this ethanol resembles inhalation anesthetic agents but differs from intravenous agents such as propofol, barbiturates, ketamine and urethane which do not affect the monosynaptic reflex (Brockmeyer and Kendig, 1995; Jewett et al., 1992; Kendig et al., 1994; Savola et al., 1991). We have previously shown that the monosynaptic reflex is sensitive to the AMPA/kainate receptor antagonist CNQX but not to the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (AP-5) (Woodley and Kendig, 1991). Thus depression of the monosynaptic reflex implies an action on either impulse initiation or non-NMDA glutamate receptor-mediated synaptic transmission. To distinguish between these possibilities we employed the large population EPSP that can be observed 1–2 segments away from the stimulating electrode, unaccompanied by a compound action potential. This large depolarization has a latency associated with monosynaptic transmission and probably reflects the spread of primary afferent terminals to motor neurons several segments from their primary targets. The results with the population EPSP confirm that glutamate receptor-mediated synaptic transmission is depressed by ethanol sufficiently to account for ethanol's depressant effects on the monosynaptic reflex.

The population EPSP is composed of an early AMPA and a smaller later NMDA receptor-mediated component. Ethanol depresses both; however, the NMDA receptor response is more sensitive under the conditions in which it was evoked in isolation from the large depolarization associated with the AMPA receptor response. NMDA-evoked responses are inhibited by ethanol at concentrations associated with intoxication but below those which cause general anesthesia in several preparations (Dildy-Mayfield et al., 1996; Lovinger et al., 1989, 1990; Morrisett and Swartzwelder, 1993; Peoples and Weight, 1995). Some studies suggest that glutamate non-NMDA receptors are also affected at modest ethanol concentrations (Dildy-Mayfield et al., 1996; Morrisett and Swartzwelder, 1993), but other studies report only modest inhibition at high concentrations (Weight, 1992). The present study suggests that in spinal cord ethanol at concentrations in the intoxicating to general anesthetic range inhibits AMPA receptor-mediated synaptic transmission as well as NMDA receptor-mediated responses. Since under the conditions of the study presynaptic effects on transmitter release cannot be distinguished from postsynaptic effects on the receptor, either or both could contribute to the depression associated with ethanol.

Ethanol profoundly depressed the slow ventral root potential. The latter is a complex response related to nociceptive neurotransmission and mediated by both NMDA receptor components in its earlier and by a variety of metabotropic receptors in its later phase. The early NMDA receptor-mediated part of the response was somewhat more sensitive to ethanol than the later, but the difference was not marked. The slow ventral root potential, in particular its later components, is exquisitely sensitive to analgesic agents. Thus depression of this response by ethanol may be related to the latter's analgesic properties (Bukusoglu et al., 1993).

The dorsal root potential is a response mediated by GABA release from interneurons and acting on $GABA_A$ receptors on primary afferent nerve terminals. The in-

terneurons in turn are exited by glutamate acting on both NMDA and non-NMDA receptors. Ethanol enhances activity at $GABA_A$ receptors at relatively modest concentrations (Harris et al., 1995; Klein et al., 1995; Leidenheimer and Harris, 1992), and the ability of alkanols to enhance $GABA_A$ function has been correlated with their general anesthetic potencies (Dildy-Mayfield et al., 1996; Mihic et al., 1994). An important role for GABA receptor enhancement in anesthesia is proposed for inhalation general anesthetics and some intravenous agents with known sites on the GABA receptor such as propofol, barbiturates, and benzodiazepines (Franks and Lieb, 1994; Tanelian et al., 1993). However, in the spinal cord when the dorsal root potential is evoked via the native circuitry rather than by direct application of a $GABA_A$ receptor agonist, the effect of ethanol as well as that of inhalation agents (Kendig and Gibbs, 1994) is depression. This is in contrast to propofol, barbiturates (Jewett et al., 1992) and benzodiazepines (Siarey et al., 1994) which enhance both directly evoked GABA responses and responses evoked by dorsal root stimulation. Similar depressant effects on inhibitory transmission to motor neurons in spinal cord (Takenoshita and Yoshiya, 1994) and to hippocampal neurons (Perouansky et al., 1996) have also been reported. The depressant effects on the circuitry in the present study can be due to inhibition of glutamate receptors on the interneurons and/or to a non-specific inhibition of inhibitory as well as excitatory neurotransmitter release. The observation that general anesthetic agents inhibit rather than enhance $GABA_A$ receptor-mediated inhibition when the response is evoked by normal neurotransmission leads to questions about the commonly assumed role of enhancement of GABA inhibition in general anesthesia.

The marked age dependence of ethanol anesthetic concentrations in contrast to the more limited age dependence of volatile clinical anesthetics has important implications for theories of anesthesia based on correlations with potency. Two reasons for age-dependent potency may be considered, one based on receptor-specific actions and the other on the as yet poorly defined phenomenon of acute tolerance.

If general anesthesia as defined by immobility is due to anesthetic actions on a limited number of receptors and ion channels, then age dependence per se implies that the representation of the anesthetically critical ion channels is different in adult and neonatal spinal cord. Moreover, under this hypothesis the difference in the degree of age dependence between ethanol and other general anesthetic agents implies that ethanol acts on a combination of receptors not completely congruent with those responsible for anesthesia by clinical inhalation agents. Information on populations of receptor subtypes in spinal cord is still fragmentary for both adults and neonates. Messenger RNA is expressed in adult spinal cord for glutamate (AMPA) receptor subunits 1–4 and glutamate NMDA receptor subunits 1–2. Glutamate kainate receptor subunits 5 and 7 are expressed in scattered cells but 6 appears to be absent (Tolle et al., 1993). Immunohistochemical localization of $GABA_A$ subunits has identified $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$, $\beta 2,3$ and $\gamma 2$ in adult rat (Bohlhalter et al., 1996). Ontogenetically GABA receptor subtypes and functional properties change from embryo to adult (Ma et al., 1993); expression of messenger RNA for subunits $\alpha 2$, $\alpha 3$, $\beta 3$ and $\gamma 2$ peaks around birth and declines toward adult levels after postnatal day 7. The $\alpha 4$, $\alpha 5$, $\beta 1-2$, $\gamma 1$ and $\gamma 3$ subunit messenger RNAs are detectable in the first 2 weeks but disappear thereafter. The spinal cord contains many other receptors and ion channels that might be linked to general anesthesia, but there is little evidence on maturational changes.

An alternative possible explanation for age difference in sensitivity is based on the concept of acute tolerance. Tolerance implies that two effects determine the apparent potency of a drug: an effect with a rapid onset, such as opioid analgesia or ethanol anesthesia, and a more slowly developing effect in the opposite direction. Determinations made after the second effect has achieved some magnitude will suggest the agent has a lower potency than when only the first operates. It has recently been suggested that for ethanol the initial sensitivity as measured by loss of righting reflex actually decreases with age (Silveri and Spear, 1996), and that the apparent lower potency of this agent in young rats, measured by time to regain righting reflex, is due to a decrease in the magnitude of acute tolerance with age (Little et al., 1996; Silveri and Spear, 1996). In this respect, it is interesting to note that neonatal ethanol anesthetic potency is correlated with the concentration that depresses the monosynaptic reflex.

The results of the present study do not provide direct support for one explanation versus the other. With particular reference to acute tolerance, effects of ethanol on all responses were time-dependent in a monotonic fashion and there was no evidence of a decrease in ethanol effect after steady state was reached in approximately 20 min. However, the pharmacokinetics of ethanol applied to the surface of the superfused isolated cord are limited by diffusion. If the development of tolerance occurs over a time span of minutes, then ethanol concentrations at the sites of action would have been rising simultaneously with tolerance development, masking a time-dependent decrease in potency. We have previously observed a manifestation of acute tolerance to opioids without a time-dependent potency decrease, accounted for by the pharmacokinetics of diffusion in the isolated cord (Feng and Kendig, 1995b).

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