AN INHIBITORY EFFECT OF ETHANOL ON ADRENERGIC NEUROMUSCULAR TRANSMISSION IN THE GUINEA-PIG VAS DEFERENS

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Abstract—Effects of ethanol on adrenergic neuromuscular transmission were investigated in the isolated vas deferens of the guinea-pig. The contractile responses to adrenergic nerve stimulation were depressed by ethanol in a concentration-dependent, reversible manner at a concentration range between 25 and 500 mM. Ethanol also depressed the contractions induced by exogenous noradrenaline. The resting membrane potentials recorded intracellularly from the smooth muscle cells were not affected by the alcohol. The excitatory junction potentials (EJPs) evoked by nerve stimulation decreased in amplitude, but the facilitation phenomena observed with repetitive stimulation remained unaltered. Ethanol slightly increased the frequency of the spontaneous EJPs, but decreased their amplitude. The extracellularly recorded action potentials from the small sympathetic nerve bundles innervating the vas deferens were suppressed by high concentrations of ethanol (more than 200 mM). These results indicate that ethanol inhibits adrenergic neuromuscular transmission in the vas deferens probably through depressing the sensitivity of the postsynaptic membrane to the transmitter and a block of axonal conduction in the presynaptic nerve terminals.

There have been many studies on the action of ethanol on synaptic transmission and neuronal excitability. In regard to the effect of alcohol on the adrenergic neuromuscular transmissions, however, experimental results are still in controversy. It has been reported that ethanol increases the spontaneous noradrenaline release from the rat vas deferens (1), but it decreases the stimulated release of noradrenaline from the rabbit cardiac sympathetic nerves (2, 3). Recently, Gillespie et al. (4) have shown that ethanol reduces the motor response to adrenergic nerve stimulation in the rat anococcygeus muscle. Despite these apparent effects on the adrenergic systems, there have been little electrophysiological data on the direct cellular effects of ethanol.

Materials and Methods
Male albino guinea-pigs, weighing between 250 and 400 g, were stunned and bled to death. The vasa deferentia were removed together with adjacent mesentery containing branches of the hypogastric nerve. Longitudinal strips of 10 mm length were prepared from 2/3 of the prostatic side. The organ bath was perfused at a constant rate of 2 ml/min with Krebs solution of the following composition (in mM): NaCl, 121.9; KCl, 4.7;
CaCl₂, 2.5; MgCl₂, 1.2; KH₂PO₄, 1.2; NaHCO₃, 15.5; and glucose, 11.5. The high-K solution was made by substituting NaCl with an equimolar amount of KCl. The solutions were gassed with 95% O₂ and 5% CO₂. The temperature was maintained at 35±1 °C, and the pH was adjusted to 7.2-7.4. Drugs were applied to the tissue by changing the inflow to the organ bath from normal Krebs solution to Krebs containing the appropriate drug, without alteration of flow rate. The time taken for 90% replacement of the contents of the bath was approx. 4 min.

Contractions of the smooth muscle were recorded isometrically with a mechano-electric transducer (Shinko Tsushin, UL-10). For the transmural postganglionic nerve stimulation, parallel silver wire electrodes were placed at the prostatic end of the vas deferens close to the entry of the hypogastric nerve. Short trains of rectangular pulses (20 pulses at 10 Hz) of 0.1-0.5 msec in duration were delivered every 40 sec from an isolator stimulator unit (WPI, 1830). The stimulus voltage was usually between 3 and 10 V. By this stimulation, rapid and large contractions with constant amplitude were repeatedly introduced.

The membrane potentials of smooth muscle cells near the serosal surface were measured with intracellular microelectrodes filled with 3M-KCl and having resistances of between 30 and 60 MΩ. The electrode was connected to a preamplifier with high input impedance (Nihon Kohden, MEZ-8101) and then led to a conventional DC- and an AC-amplifier (time constant, 0.2 sec). A low-gain DC potential and a high-gain AC potential were simultaneously displayed on an oscilloscope and photographed. Only those cells which had a resting membrane potential of over 50 mV were used for the experimental analysis. In order to avoid dislocation of the microelectrode, the stimulus frequency and stimulus voltage were adjusted in each preparation so that facilitated excitatory junction potentials (EJPs) were less than 15 mV in amplitude (below the threshold for initiation of spikes).

For investigation of electrical events occurring in prejunctional nerve terminals, action potentials from the sympathetic nerve bundles were recorded extracellularly using glass microelectrodes filled with 3M-KCl and having resistances of less than 3 MΩ (5). The time constant of the recording system was 3 msec. In this series of experiments, a preset number of responses (usually 10 at a stimulation frequency of 0.5 Hz) were digitized (up to 10 μsec/point) and averaged on a signal averager (Nihon Kohden, ATAC 350).

Drugs used were noradrenaline hydrochloride, acetylcholine chloride, hexamethonium bromide, atropine sulfate, tetrodotoxin, phentolamine methanesulfonate, phenoxybenzamine hydrochloride, guanethidine sulfate, and ethyl alcohol (ethanol). All data have been expressed as the mean±standard errors.

Results

Effects on contractions of the vasa in response to transmural electrical stimulation: The twitch responses of the vas deferens to transmural electrical stimulation were reduced in amplitude in the presence of ethanol (Fig. 1). This effect of ethanol was concentration-dependent. The minimum effective concentration was approx. 25 mM. At the moderate doses of ethanol, the reduction of the twitch contractions progressively developed and reached a peak between 7 and 10 min after superfusion of the substance. Therefore, the changes of the mechanical activities were usually determined 10 min after introduction of ethanol. At 500 mM, the highest concentration tested in this study, the alcohol completely blocked the neurally evoked contraction within 5 min. The ethanol-
induced depression was quickly reversed on washing the organ bath free of the drug and could be repeated several times in any one of the preparations. The twitch contraction was not modified by 10^{-5} g/ml hexamethonium, but it was completely eliminated by 10^{-6} g/ml tetrodotoxin. In the presence of either hexamethonium or atropine (5\times10^{-6} g/ml), the depression of the twitch contraction by ethanol was observed to be similar to that in the control experiment. The alcohol had no discernible effect on the resting tension after complete blockade of neuromuscular transmission by tetrodotoxin.

Phentolamine, at a relatively low concentration (5\times10^{-5} g/ml), increased the nerve evoked contractions. Phenoxybenzamine at a concentration of 10^{-6} g/ml produced the same results. The alcohol-induced inhibition, however, was unaltered even in the presence of phentolamine or phenoxybenzamine. A high concentration of phentolamine (10^{-4} g/ml) resulted in complete abolition of contractions, and the muscle relaxed to resting tension level even though the stimulus intensity was increased up to 100 V. Similar results were obtained with guanethidine (10^{-6} g/ml). Ethanol, when applied in the presence of a high concentration of phentolamine or guanethidine, produced no observable change in tension.

Effects on contractions induced by noradrenaline and acetylcholine: Noradrenaline and acetylcholine similarly produced initial large and rapid contractions and succeeding slow contractions. Ethanol decreased these contractions both in their peak amplitudes and in their durations. The dose-response curves demonstrate that ethanol decreased the sensitivity not only to noradrenaline, but
also to acetylcholine (Fig. 2). A high-K solution also produced an initial phasic and an ensuing tonic contraction. In our preparation, however, the tonic component was very small (6-8). Ethanol did not appreciably affect the K-induced contraction even at the highest concentration tested (Fig. 2C).

**Effects on the resting membrane potentials:** Intracellular membrane potentials were recorded in 25 cells by stable impalements of microelectrodes over 30 min. The mean value of the resting membrane potentials (RMP) during the control period was $-56.1 \pm 1.6$ mV. As shown in Fig. 3, application of ethanol produced no significant change in the RMP. In a few cells (3 out of 25), the alcohol induced a weak hyperpolarization by less than 5 mV at a dose of 200 mM, which lasted about 5 min and then returned gradually to the original potential, even though the drug Fig. 3. Effects of EtOH on the resting membrane potentials (RMP) and excitatory junction potentials (EJPs) recorded intracellularly from the smooth muscle cell of the vas deferens. Repetitive transmural stimulation was applied at 0.2 Hz with rectangular pulses of 0.5 msec in duration and 7 V in strength. Upper tracings (a): DC-records, lower tracings (b): AC-records (time constant, 0.2 sec).
infusion continued. Calculations of the mean values, however, revealed that there was no statistically significant effect of ethanol on the RMP in the concentration range between 50 and 400 mM.

**Effects on the evoked EJPs:** The excitatory junction potentials (EJPs) were evoked by repetitive transmural stimulation at a frequency of 0.2 Hz (Figs. 3 and 4). During the initial 5 to 7 impulses of a train, successive EJPs were facilitated and then reached a steady state level. After a pause of several minutes, the same facilitation phenomena could be repeated in the same cell. During superfusion of ethanol, these evoked EJPs considerably decreased in amplitude. The time course of the change in EJPs was nearly parallel with that of the mechanical change at a given concentration of ethanol (Figs. 1 and 3). The mean amplitude of facilitated EJPs produced by continuous stimulation at 0.2 Hz was 10.1±0.7 mV during the control period, and it was reduced to 6.0±0.6 mV after 200 mM ethanol. The first EJP evoked by a train of impulses was also depressed by alcohol. The percentage reductions in the amplitudes of the first and the facilitated EJPs were 43±7% and 40±4%, respectively, after 200 mM ethanol (Figs. 3 and 4). Figure 4 further demonstrates that though each EJP was decreased in amplitude, the facilitation was not appreciably affected by ethanol (9, 10).

**Effects on the spontaneous EJPs:** The spontaneous miniature EJPs (min. EJPs) were recorded in 8 cells from 3 preparations. Since the small min. EJPs with amplitudes less than 0.5 mV were difficult to distinguish from baseline noise fluctuation, only those

![Fig. 4. Effects of EtOH on the facilitation of successive EJPs during a train of stimuli. A: Specimen records. The upper panel (1) was obtained just before the drug administration; the lower (2), 10 min after the EtOH superfusion (200 mM) was started. In graph B, the ordinate represents amplitudes of facilitated EJPs during a train of 10 stimuli at 0.2 Hz. Each point gives the mean of five experiments. •: before, ○: after 200 mM EtOH. In C, the ordinate represents the magnitude of facilitation expressed in the form \((V_n - V_0)/V_0\), where \(V_0\) is the amplitude of the first EJP of the train and \(V_n\), the amplitude of the \(n\)th EJP. The abscissa: time course in sec.](image)

![Fig. 5. Effects of EtOH on the spontaneous EJPs (min. EJPs) of the vas deferens. Amplitude histograms before (1) and during application of 200 mM EtOH (2). Specimen records are shown in each graph.](image)
with amplitude of at least 1 mV were counted. As has been illustrated in Fig. 5, ethanol slightly increased the frequency of the min. EJPs, while it decreased their amplitudes. For all cells studied, the mean amplitudes under the control condition and in the presence of 200 mM ethanol were 2.51±0.14 mV (N=149) and 1.97±0.07 mV (N=175), respectively. The mean frequency was increased from 7.10±0.16/min to 8.31±0.13/min.

Effects on sympathetic nerve action potentials: Compound action potentials were recorded extracellularly from the sympathetic nerve bundles which innervate the vas deferens. Several monophasic and biphasic potentials were induced in succession by a single stimulus pulse applied to the sympathetic nerves near the prostatic end of the vasa (Fig. 6). The estimated conduction velocity was in the range between 0.5 and 1.7 m/sec. The potentials were completely eliminated by tetrodotoxin, but not by hexamethonium. Thus, they were confirmed to be originated by the postganglionic C fibers. Ethanol, at the concentration of more than 200 mM, decreased or abolished the compound action potentials (Fig. 6). At a concentration range between 50 and 100 mM, the alcohol had no effect on these potentials.

Discussion

In the present experiments, the twitch contraction induced by transmural electrical stimulation was blocked by appropriate concentrations of phentolamine, guanethidine and tetrodotoxin, but was not blocked by hexamethonium and atropine. Thus the contraction can be considered as a response due to excitation of the postganglionic adrenergic nerve. Ethanol caused a reversible, concentration-dependent depression of the neurally evoked contractions. This effect was not modified by hexamethonium and atropine, showing that the action of alcohol was not mediated by a cholinergic mechanism in the adrenergic nerve terminals (2, 3). The twitch contraction was enhanced by α-adrenoceptor blocking agents at relatively low concentrations. It has been postulated that α-receptor blockades greatly increase the noradrenaline output per stimulus, and the presynaptically located α-receptor forms part of a negative feedback mechanism for the transmitter release (11-14). Ethanol still depressed the twitch contraction even in the presence of these agents. It is assumed, therefore, that the alcohol does not affect the presynaptic α-adrenergic mechanism.

Ethanol also depressed the contractile responses to noradrenaline and to acetylcholine. Since the same dose of alcohol did not alter the phasic contraction induced by high-K solution, it seems unlikely that the substance directly inhibits the contractile mechanism itself. These results rather suggest that ethanol may have little effect on the
potential-dependent ion channels which can be activated by high-K solution, but affect mainly the receptor-operated ion channels which can be stimulated by noradrenaline and acetylcholine (3, 15). Ethanol has been reported to inhibit the contractile responses of the vascular smooth muscles to several vasoactive agents including catecholamines (16–18). Though the ionic basis of the ethanol action in the vasa deferentia is yet obscure, it is assumed that the alcohol depresses the transmitter action on the postsynaptic smooth muscle membrane (4). This assumption is also supported by the fact that not only neurally evoked EJPs but also min. EJPs were decreased in amplitude by superfusion of ethanol, while the resting membrane potential remained unchanged.

A presynaptic mechanism may be also involved in the reduction of the evoked EJPs. We found that high ethanol concentrations decreased or abolished the compound action potentials recorded from the postganglionic sympathetic nerve bundles. Many studies of in vitro preparations have shown that ethanol decreased the size of the action potential, depresses excitability, and thus blocks the propagation of action potentials in the peripheral nerves (19–21). The alcohol probably decreases the size of the spike potentials in the sympathetic nerve terminals, especially in the varicose region. Consequently, it may cause a decrease in transmitter release in response to transmural electrical stimulation. Gothert et al. (3) reported that in the isolated rabbit heart, the noradrenaline release induced by electrical stimulation was depressed by high concentrations of ethanol. Gillespie et al. (4) have also demonstrated that ethanol inhibits the motor response in the rat anococcygeus muscle to field stimulation of the adrenergic nerves. They suggest that a high concentration of ethanol (more than 400 mM) exerts a local anesthetic action on nerve fibers. Because the facilitation of the EJPs produced by a train of impulses was unaltered after ethanol administration, the substance seems to have no effect on mobilization of the transmitter which takes place during successive nerve impulses (9, 10).

The mean frequency of the min. EJPs slightly increased during ethanol superfusion. Since the small min. EJPs with amplitude of less than 1 mV were not counted in this analysis and further more, since the amplitude of the min. EJPs was reduced by ethanol, calculations in the mean frequency after ethanol may be slightly underestimated. Thus it is possible that the alcohol increases the spontaneous transmitter release from the sympathetic nerves (1).

From the results described above, it may be concluded that ethanol depresses the adrenergic neuroeffector transmission in the guinea-pig vas deferens, probably through a depression of the sensitivity of the postsynaptic membrane to transmitter and a block of axonal conduction in the sympathetic nerve ending.

References

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