SELECTIVE EFFECT OF ETHANOL ON THE VESTIBULAR NUCLEUS NEURONS IN THE CAT

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Abstract—Effects of intravenous administration of ethanol on the neuronal activities of the lateral vestibular nucleus (LVN) and spinal trigeminal nucleus (STN) were investigated in cats. The LVN neurons were classified into three groups according to the latency of the first spike elicited by orthodromic vestibular nerve stimulation and antidromic vestibulospinal tract stimulation: monosynaptic, polysynaptic I and polysynaptic II neurons. Ethanol of 0.2-1.6 g/kg dose-dependently suppressed the orthodromic spike generation of the monosynaptic and polysynaptic II neurons without affecting their latency and antidromic spike generation of the former neuron. The mean spike numbers of the monosynaptic and polysynaptic I I neurons were significantly decreased with ethanol over 0.4 g/kg. The polysynaptic I neuron, however, remained unaffected by the drug up to 0.8 g/kg. Similarly, the spike generation of the STN relay neuron and interneuron elicited by trigeminal nerve stimulation remained unaltered with ethanol given in doses up to 0.8 g/kg. These results indicate that small doses of ethanol more selectively interfere with synaptic transmission in the LVN monosynaptic and polysynaptic II neurons than transmission in the STN relay neurons and interneurons.

There are numerous reports on the different effects of ethanol on neuronal activities in the central nervous system of experimental animals. Ethanol depressed monosynaptic and polysynaptic reflex discharges in the spinal cord, while the drug produced an increase in Renshaw cell activity with motor nerve stimulation (1, 2). Both inhibitory and excitatory effects of ethanol on spontaneous firing of single neurons in the lateral hypothalamus, zona incerta, thalamus, cerebral cortex and locus coeruleus have been observed (3–6). Ethanol was found to inhibit single units in the hippocampus (7), whereas in the lateral geniculate nucleus, relative resistance to the drug has been reported (8). Regional and dose-dependent differences in the effects of ethanol have been demonstrated in multi-unit activity recorded from various areas of the brain (9).

It has been well documented that ingestion of even low doses of ethanol produces equilibrium disorders and nystagmus in humans (10). Thus, low doses of ethanol may selectively affect neuronal activity in the vestibular nuclei and cerebellum. Actually, Eidelberg et al. (11) reported that most of lateral vestibular nucleus (LVN) neurons and Purkinje cells are depressed by relatively low doses of ethanol, however, these observations were based on only spontaneous firing of the neurons. We attempted to determine whether

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or not a small amount of ethanol could selectively affect the spikes of LVN neurons elicited by vestibular nerve stimulation, in comparison with the effect of ethanol on spikes of the spinal trigeminal nucleus (STN) neurons, as evoked by trigeminal nerve stimulation.

MATERIALS AND METHODS

Forty-five adult cats of both sexes weighing 2.5-4.0 kg were used. All surgical procedures were carried out with the animal under ether inhalation anesthesia. After cannulating the trachea and femoral vein, the left tympanic bulla was trepanned under a ventral approach. A bipolar stainless steel electrode for vestibular nerve stimulation was inserted through the exposed round window and immobilized with dental cement. The head of the animal was fixed in a stereotaxic instrument, and the occipital skull and bony tentorium were removed to allow for insertion of the recording electrodes. A concentric bipolar electrode for antidromic stimulation was introduced into the ipsilateral vestibulospinal tract located 2.5 mm from the midline and 3.0 mm from the spinal cord surface at C2-C3 level, according to the method of Rapoport et al. (12). In the experiments on STN neurons, small holes were made on the skull for insertion of bipolar electrodes into the left trigeminal nerve trunk (A: 6.0, L: 6.0, H: -10.0) for orthodromic stimulation and the right medial lemniscus (A: 4.0, L: 5.5, H: -0.5) for antidromic stimulation, according to the brain map of Snider and Niemer (13). After all surgical procedures had been completed, the animal was anesthetized with α-chloralose (30 mg/kg i.v.) and immobilized with gallamine triethiodide (5 mg/kg/hr i.v.). Respiration was maintained with an artificial respirator. All wound edges and pressure points were locally anesthetized with 8% lidocaine spray, repeatedly throughout the experiments. Body temperature was kept at 36.5-37.5°C by means of a heating pad placed beneath the animal. In several animals, femoral blood pressure was monitored using a pressure transducer.

Stimuli composed of square wave pulses with 0.05 msec duration and less than 10 V in intensity were applied every 1.6 sec. The stimulus used was 1.5 times higher than the threshold voltage in intensity. The threshold stimulus was of minimal intensity in voltage and consistently produced at least one spike with each stimulation. A glass-insulated silver wire microelectrode with an electrical resistance of approx. 1 MΩ was inserted to record the firing of single neurons in the left LVN (P: 8.0, L: 4.0, H: -3.0 to -4.0) and STN (P: 9.0, L: 5.5, H: -5.0 to -6.0). This type of microelectrode allowed for simultaneous recording of the field potential and single neuron activity. The responses were amplified and displayed on an oscilloscope (Nihon Kohden, VC-9). Evidence for the spontaneous firing of the LVN neurons was stored on magnetic tape, and then recorded on X-Y plotter after a computer analysis (Nihon Kohden, ATAC-350).

Ethanol (25%, w/v) dissolved in saline and administered i.v. in cumulative doses was increased logarithmically at 10-min intervals. Recording of the responses was started 5 min after each injection, and at least 10 successive responses were photographed with a long recording camera. Student's t-test was used to determine the significant differences of the mean of 10 successive responses in each neuron and of the mean values of 6-7 pre-
parations tested before and after the application of ethanol. After termination of the experiments, recording sites were marked by passing a direct current of 20–30 μA for 15–25 sec, and histologically checked using cresyl violet stain. Figure 1A and B demonstrate the sites of recording electrodes in the LVN and STN.

RESULTS

1) Lateral vestibular nucleus neurons: The field potential of LVN elicited by vestibular nerve stimulation consisted of pre-, mono- and polysynaptic components, as already designated by Shimazu and Precht (14). The latencies of these three components were approx. 0.5, 1.1 and 2.4 msec, respectively. According to the latency of the first spike activated by orthodromic stimulation of the vestibular nerve, the single neurons in the LVN were classified into three groups: monosynaptic, polysynaptic I and polysynaptic II neurons. The monosynaptic neuron produced spikes on the monosynaptic component of field potential with latencies ranging 1.0–1.5 msec upon the orthodromic stimulation (Fig. 2A). The mean latency of 30 monosynaptic neurons was 1.3 ± 0.1 (S.E.) msec. The monosynaptic neurons were activated by antidromic stimulation of the vestibulospinal tract with a mean latency of 1.1 ± 0.1 msec, and the antidromic spike consistently followed a high frequency stimulus up to 200 Hz (Fig. 2B and C). The first spike of polysynaptic I neuron upon vestibular nerve stimulation was observed on the polysynaptic component of field potential and the mean latency of 23 polysynaptic I neurons was 2.8 ± 0.2 msec (range: 2.1–4.1 msec) (Fig.
3A). The spike latency of the polysynaptic I neurons varied somewhat with each stimulus, compared with a relatively consistent latency of the monosynaptic neurons. The poly-

**Fig. 2.** Effects of ethanol on orthodromic (A) and antidromic spikes (B) of monosynaptic neuron in the lateral vestibular nucleus. The responses are recorded before and 5 min after 0.4 and 1.6 g/kg of ethanol. C: superimposed antidromic spikes of the same neuron by high frequency stimulation of 200 Hz. Solid triangles indicate the stimulus artifacts to the vestibular nerve (A) and vestibulospinal tract (B). Calibration: 0.5 mV, 5 msec.

**Fig. 3.** Effects of ethanol on spikes of polysynaptic I (A) and polysynaptic II (B) neurons in the lateral vestibular nucleus. The responses are recorded before and 5 min after 0.4 and 1.6 g/kg of ethanol. Solid triangles indicate the stimulus artifacts to the vestibular nerve. Calibration: 0.5 mV, 5 msec.

**Fig. 4.** Spontaneous firing pattern of two monosynaptic (A and B) and two polysynaptic I (C and D) neurons. Abscissa indicates time and ordinate shows number of spikes/sec.
synaptic II neurons responded to vestibular nerve stimulation with latencies longer than 9.0 msec, and the mean latency of 7 polysynaptic II neurons was 20.1±3.8 msec (Fig. 3B). Neurons that could be classified by these criteria were used to determine the effects of ethanol.

An almost total lack of spontaneous firing was observed in 9 out of 12 monosynaptic neurons in the LVN, and the remaining 3 neurons showed sporadic and irregular discharges of the spontaneous firing pattern (Fig. 4A and B). In contrast, all 7 polysynaptic I neurons tested spontaneously fired spikes, with a relatively regular pattern, and the firing rate of these neurons ranged from 10 to 40/sec (Fig. 4C and D).

2) Effects of ethanol on LVN neurons: Orthodromic spike generation of the monosynaptic neurons was dose-dependently inhibited by i.v. administration of ethanol, as demonstrated in Table 1. When 0.2 and 0.4 g/kg of ethanol were given, a significant reduction of the spike number was observed in 3 and 4 out of 7 neurons tested, respectively. The mean spike number of the 7 neurons was significantly (P<0.05) reduced to 1.3±0.2 with 0.4 g/kg of ethanol from 2.1±0.2 before the drug administration. There were no alterations of the latency of the first spike in all 7 neurons examined. In contrast to the orthodromic spike generation, the antidromic spike generation of the monosynaptic neurons upon vestibulospinal tract stimulation remained unaltered after ethanol administration up to 1.6 g/kg. The mean antidromic spike numbers of 7 neurons were 1.0±0.1 and 1.0±0 before and 5 min after 1.6 g/kg of ethanol, respectively.

Unlike the monosynaptic neurons, firing of the polysynaptic I neurons upon vestibular nerve stimulation was scarcely affected by ethanol up to 0.8 g/kg (Table 1). Increasing the dose to 1.6 g/kg resulted in an inhibition of spike generation in 4 out of 7 neurons examined, however, the mean spike number of all 7 neurons tested was not significantly decreased, even with a dose of 1.6 g/kg. Spike number of the polysynaptic II neurons elicited by vestibular nerve stimulation was dose-dependently reduced by ethanol, while the spike latency was little affected (Table 1). A significant reduction in the spike number was observed

| Table 1. Effects of ethanol on spike generation of lateral vestibular nucleus neurons upon vestibular nerve stimulation |
|---------------------------------------------------------------|
| **Ethanol (g/kg i.v.)** | 0 | 0.2 | 0.4 | 0.8 | 1.6 |
|-------------------------|---|-----|-----|-----|-----|
| **Monosynaptic neurons (n=7)** | 2.1±0.2 | 1.6±0.2 | 1.3±0.2* | 1.2±0.2** | 0.9±0.2** |
| (3/7)** | (4/7) | (6/7) | (7/7) |
| **Latency (msec)** | 1.3±0.1 | 1.3±0.1 | 1.3±0.1 | 1.3±0.1 | 1.3±0.1 |
| **Polysynaptic I neurons (n=7)** | 0.9±0.1 | 0.9±0.1 | 0.9±0.1 | 0.8±0.1 | 0.5±0.2 |
| (0/7) | (0/7) | (1/7) | (4/7) |
| **Latency (msec)** | 2.8±0.2 | 2.9±0.3 | 2.9±0.3 | 3.2±0.5 | 3.1±0.3 |
| **Polysynaptic II neurons (n=7)** | 3.2±0.7 | 2.2±0.4 | 1.5±0.2* | 0.8±0.2** | 0.1±0.1** |
| (2/7) | (5/7) | (7/7) | (7/7) |
| **Latency (msec)** | 20.1±3.8 | 22.0±3.8 | 23.2±3.6 | 26.4±3.6 |

n: Number of preparations. Each value represents the mean±standard error. Significant difference * at P<0.05 and ** at P<0.01, as compared with the value before ethanol administration. *** ( ) indicates number of animals affected against total number tested.
in 2 and 5 out of 7 polysynaptic II neurons following administration of ethanol of 0.2 and 0.4 g/kg, respectively (P<0.05). The mean spike number of 7 neurons tested was significantly decreased with ethanol in a dose of over 0.4 g/kg (P<0.05).

3) Effects of ethanol on STN neurons: Neurons in the STN were classified into relay neuron, type-A interneuron and type-B interneuron, according to the response pattern to orthodromic stimulation of the trigeminal nerve and antidromic stimulation of the contralateral medial lemniscus, as reported previously (15, 16). Briefly, the relay neuron responded to both orthodromic and antidromic stimulation (Fig. 5A and B). The mean latency of orthodromic spike of 6 relay neurons was 3.0±0.3 (S.E.) msec. The antidromic spikes were consistently elicited with a short latency of 1.2±0.2 msec, and followed a high frequency stimulus up to 200 Hz. The type-A interneuron also responded both to orthodromic and antidromic stimulation, however, the latency of antidromic spikes was not less than 2.0 msec and the antidromic spike could not follow a high frequency stimuli over 50 Hz. The type-B interneuron fired spikes upon orthodromic stimulation with a relatively long latency of 8.9±1.2 msec (n=7), but such did not occur with antidromic stimulation (Fig. 5C).

The effects of ethanol on the relay neuron and type-B interneuron were also examined. The spike generation of relay neuron with orthodromic and antidromic stimulation was not affected by ethanol in a dose up to 1.6 g/kg. The mean orthodromic spike number of 6 relay neuron tested was 4.0±1.0 and 3.9±1.3 before and 5 min after the administration of 1.6 g/kg, respectively. The mean antidromic spike number of the 6 relay neurons was 1.5±0.2 before the drug administration, and the number was also 1.54±0.2 after 1.6 g/kg of ethanol. A significant (P<0.05) inhibition of spike generation was observed in 2 and 2 out of 7 type-B interneurons when ethanol was given in doses of 0.4 and 0.8 g/kg, respectively. However, the mean spike number of 7 neurons examined was not significantly reduced with ethanol up to 0.8 g/kg. When the dose was increased to 1.6 g/kg, a significant reduction in the spike number was observed in 5 interneurons and the mean number of 7
interneurons tested was decreased to 0.7 ± 0.4 from 4.5 ± 0.9; while the mean latency was not significantly affected (Table 2).

4) Effects of ethanol on blood pressure: Maximum and minimum blood pressures recorded from the femoral artery were 120-160 and 80-120 mmHg, respectively. Ethanol up to 0.8 g/kg (i.v.) produced no apparent alterations in the blood pressure. Slight lowering of the blood pressure occurred immediately after giving 1.6 g/kg of the drug, however, the pressure returned to the control level 2-3 min after this administration (Fig. 6).

Table 2. Effects of ethanol on spike generation of spinal trigeminal nucleus neurons upon trigeminal nerve stimulation

|                     | Ethanol (g/kg i.v.) |
|---------------------|---------------------|
|                     | 0       | 0.2     | 0.4     | 0.8     | 1.6     |
| Relay neurons (n=6) | Spike number: | 4.0±1.0 | 4.2±1.1 | 4.1±1.1 | 4.2±1.4 | 3.9±1.3 |
|                     | Latency (msec):   | 3.0±0.3 | 2.9±0.3 | 2.9±0.3 | 3.0±0.3 | 3.0±0.2 |
| Interneurons (n=7)  | Spike number:     | 4.5±0.9 | 4.4±1.0 | 3.9±0.9 | 3.8±1.1 | 0.7±0.4**|
|                     | Latency (msec):   | 8.9±1.2 | 8.9±1.3 | 9.2±1.3 | 9.5±1.4 | 12.3±3.2 |

n: Number of preparations. Each value represents the mean ± standard error. * ( ) indicates number of animals affected against total number tested. Significant difference ** at P<0.01, as compared with the value before ethanol administration.

Fig. 6. Effects of ethanol (0.8 and 1.6 g/kg i.v.) on blood pressure recorded from the femoral artery.

Relatively low doses of ethanol inhibited the orthodromic spike generation of the LVN monosynaptic neuron without affecting the latency, yet had no effects on generation of the antidromic spike. These results indicate that low doses of ethanol impair the synaptic transmission of the monosynaptic neuron but do not affect axonal conduction. It has been demonstrated that acetylcholine may act as a synaptic transmitter from the vestibular nerve to the vestibular nucleus (17-19). Therefore, our findings that ethanol inhibited the ortho-

DISCUSSION

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dromic spike generation in the LVN monosynaptic neuron, suggest that the drug may interfere with the release and/or the effect of acetylcholine on the neuron. The polysynaptic I neuron was more resistant to ethanol than was the monosynaptic neuron. The former apparently does not make a direct connection with the monosynaptic neuron, because the monosynaptic neuron was antidromically excited by vestibulospinal tract stimulation. Thus, differences of sensitivity to ethanol between monosynaptic and polysynaptic I neurons may explain the differences in the transmitter or in membrane characteristics.

As previously reported (20), ethanol concentration in the blood was 87 mg/dl (n=5) 5 min after an i.v. administration of 0.4 g/kg, under the same experimental conditions. This level corresponds to the blood concentration which produces signs such as nystagmus and equilibrium disorders in humans with a low tolerance to alcohol (10, 21). Our findings that 0.4 g/kg of ethanol inhibited the transmission of the monosynaptic neuron in the LVN are in line with clinical manifestations following ingestion of moderate doses of ethanol. The vestibular neurons have been classified by Shimazu and Precht into two types (14, 22): kinetic and tonic neurons. In the kinetic neuron, spikes were elicited on monosynaptic field potential with vestibular nerve stimulation and spontaneous firing which was absent or sporadically irregular was steeply increased in response to horizontal rotation with constant angular acceleration and decreased with constant angular velocity. In the tonic neuron, spikes were produced on polysynaptic field potential and spontaneous firing was observed. The spontaneous firing rate initially increased with rotation, and then maintained a constant value during the remainder of this acceleration. It is considered from our results of the spontaneous firing pattern and the evoked spikes with vestibular nerve stimulation that the LVN monosynaptic neuron corresponds to the kinetic neuron and the polysynaptic I neuron to the tonic neuron. It is of interest, therefore, that the polysynaptic I neuron was more resistant to ethanol, thereby suggesting that the former neuron might be playing a different role in the control of posture and/or equilibrium. Since the impulses to the polysynaptic II neuron may be transmitted multisynaptically even via synapses outside the LVN, the exact site of action of ethanol on the polysynaptic II neuron remains to be defined.

In contrast to the LVN neurons, the relay neuron and type-B interneuron transmissions in the STN were not affected by ethanol given in doses up to 0.8 g/kg. This difference in sensitivity to ethanol between these neurons in the brain has been reported by others. Renshaw cells in the spinal cord were activated by low doses of ethanol, but motoneurons and spinal dorsal interneurons were inhibited by the drug (1, 2). Activity of Purkinje cells in the cerebellum was depressed by ethanol, while cerebellar interneurons were activated (11). Lateral geniculate nucleus neurons were relatively resistant to ethanol, compared with the activity of the visual cortex (8). The present findings that low doses of ethanol selectively inhibited the orthodromic spike generation of the monosynaptic and polysynaptic II neurons in the LVN but not the polysynaptic I neuron in the LVN nor the relay neuron and interneuron in the STN are in good agreement with these aforementioned observations.
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