Activation of Lateral Vestibular Nucleus Neurons by Iontophoretically Applied Phencyclidine

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Abstract—Microiontophoretic studies were performed to elucidate the effects of phencyclidine (PCP) on neuronal activity in the lateral vestibular nucleus (LVN) of cats anesthetized with α-chloralose. Spikes elicited in the monosynaptic LVN neurons by vestibular nerve stimulation were not affected by iontophoretic application of PCP up to 100 nA, but they were blocked by atropine (30-50 nA). A dose-dependent increase in spontaneous firing during application of PCP was obtained in 11 of 15 monosynaptic neurons, in all of which firing was increased by iontophoretically applied acetylcholine (ACh). Simultaneous application of atropine completely inhibited the PCP and ACh-induced increase in the firing without affecting the glutamate-induced firing. These results indicate that PCP acts on the LVN monosynaptic neurons receiving input from the vestibular nerve in a similar manner to ACh.

Phencyclidine (PCP) developed as an anesthetic for veterinary use has potent psychotomimetic activity and has been widely abused in the past decade. This compound produces a variety of effects on the central nervous system such as the central cholinergic, dopaminergic, noradrenergic, serotonergic, GABAergic and enkephalinergic systems (1-6). In acute PCP intoxication, the clinical profile includes agitation, confusion, ataxia and muscle rigidity as well as cholinergic signs such as profuse diaphoresis, bronchospasm, miosis and hypersalivation (7, 8). In addition, nystagmus in both vertical and horizontal directions is one of the hallmarks of the intoxication (9-11).

Since electrophysiological studies have demonstrated that the vestibular nuclei are one of the sites responsible for producing nystagmus (12), microiontophoretic studies were carried out to elucidate the effects of PCP on neuronal activity of the LVN, which is assumed to be partly involved in the occurrence of nystagmus.

Materials and Methods

Fifteen adult cats of either sex weighing 2.5–3.5 kg were used. Surgical procedures were performed under ether anesthesia. The trachea and femoral vein were cannulated, and a bipolar stainless steel electrode was inserted into the vestibular nerve located in the round window of the middle ear cavity and fixed there. After fixation of the animal's head in a stereotaxic instrument, part of the occipital cranium and part of the bony tentorium were removed to allow insertion of the recording electrode. Thereafter, ether anesthesia was replaced with α-chloralose (30 mg/kg, i.v.) with supplemental doses of 10 mg/kg as required, and the animal was immobilized with gallamine triethiodide (5 mg/kg/hr, i.v.) under artificial respiration. All wound edges and pressure points were locally anesthetized with 8% lidocaine spray repeatedly throughout the experiment. Body temperature was maintained at 37.0-37.5°C with a heating pad.

The neuronal activity in the left LVN (P:
8.0, L: 4.0, H: -3.5 to -4.5) (13). extracellularly recorded using a glass-insulated silver wire microelectrode (with an electrical resistance of approx. 1 MΩ) attached along a seven-barreled micropipette, the outer diameter of which was approximately 10 μm. The distance between the tips of the recording microelectrode and the micropipette was within 20 μm. Each pipette was filled with either 10 or 50 mM PCP HCl, 1.0 M ACh chloride (Dai-ichi Seiyaku Co.), 0.2 M atropine sulfate (Sigma), 1.0 M monosodium L-glutamate (Sigma) and 3.0 M NaCl for checking the current effect. These chemicals were iontophoretically applied to the immediate vicinity of the target neuron using a microiontophoresis programmer (WP-I, Model 160) with a positive current except for glutamate which was ejected with a negative current.

The stimulus applied to the vestibular nerve ipsilateral to the recording site every 1.6 sec was a square wave pulse of 0.05 msec duration and 1–10 V, which was 1.5 times more intense than the threshold voltage. The spikes of the LVN neurons upon the nerve stimulation were displayed on an oscilloscope (Nihon Kohden, VC-9), and 10–30 successive responses were photographed. A poststimulus latency histogram of 30 successive responses was simultaneously obtained using a computer (Nihon Kohden, ATAC-350) and an XY-plotter. Spontaneous firing was continuously recorded on an ink-writing recorder (Nihon Kohden, RJG-4022) through a spike counter (Dia Medical System, DSE-325p). Statistical significance of the data was determined by Student's t-test. Further details of the procedures have been described previously (14–16).

**Results**

**Effects on monosynaptic spikes:** The stimulus applied to the vestibular nerve produced a field potential composed of P, N₁, and N₂ waves corresponding to the pre-, mono- and polysynaptic components, respectively (17). According to the firing pattern and latency of the first spike elicited by the nerve stimulation, the LVN neurons were classified into two groups: monosynaptic and polysynaptic neurons. In the former neuron, the spike was superimposed on the N₁ wave of the field potential with a consistent latency of less than 1.4 msec (Fig. 1A); and in the latter, the spike appeared on the N₂ wave with relatively dispersed latencies, as described previously (15, 18). The present studies were undertaken on the monosynaptic neurons, because ACh is considered to be involved in the afferent transmission to the monosynaptic neuron (16, 18). The mean spike latency of 24 monosynaptic neurons was 1.14±0.07 (S.E.) msec.

Iontophoretic application of PCP up to 100 nA did not have any apparent effects on the spike generation induced by vestibular nerve stimulation in the monosynaptic neurons (Fig. 1B). However, higher doses of PCP (200 nA) sometimes reduced the spike height without affecting the latency or number of spikes upon the stimulation (Fig. 1C). The poststimulus latency histogram in Fig. 2 also demonstrates that PCP had no significant effects on the spike number or latency upon the nerve stimulation, while atropine at a dose of 30 nA inhibited the spike generation. When PCP at doses of 100–150 nA was applied for 60 sec, the spike number of 24 monosynaptic neurons upon vestibular nerve stimulation was 1.25±0.08, while it was 1.27±0.09 before application of PCP. The mean latencies of the first spike of these neurons were 1.13±0.07 and 1.14±0.07 msec in the presence or absence of PCP, respectively.

**Effects on spontaneous firing:** The effects of PCP were examined on spontaneous firing of the LVN neurons, which were monosynaptically activated by vestibular nerve stimulation. These neurons exhibited either very few spontaneous firings of less than 1/sec or an irregular and grouping discharge pattern, as previously reported (15). The effects of PCP were studied in the former neurons with rare spontaneous firing. Iontophoretic application of PCP produced a dose-dependent increase in firing of the neurons, in which ACh and glutamate also increased the firing (Fig. 3). There was a good correlation between the effects of ACh and PCP (Table 1). An increase in the firing rate during iontophoretic application of ACh
was observed in 15 of 44 monosynaptic neurons. In 11 of the 15 neurons activated by ACh, the firing was also increased with iontophoretically applied PCP up to 120 nA. In addition, the firing was not affected by PCP in 27 of 28 neurons unresponsive to

Fig. 1. Effects of phencyclidine (PCP) on spikes elicited by vestibular nerve stimulation in a monosynaptic neuron in the lateral vestibular nucleus. A, control; B and C, 1 min after the onset of iontophoretic application of PCP at doses of 100 and 200 nA, respectively; D, 3 min after the cessation of PCP (200 nA) application. In each row, responses were serially recorded every 1.6 sec. Solid triangles indicate stimulus artifacts. Arrows in C indicate a reduction of spike height induced by PCP (200 nA).

Fig. 2. Poststimulus latency histogram obtained from 30 successive responses of a lateral vestibular nucleus neuron monosynaptically elicited by vestibular nerve stimulation. A, control; B and C, during iontophoretic application of phencyclidine (PCP, 50 nA) and atropine (30 nA), respectively. Actual spikes are shown on the right side. A solid triangle indicates stimulus artifacts.
Fig. 3. Effects of iontophoretic application of glutamate (G), acetylcholine (A) and phencyclidine (P) on spontaneous firing of a lateral vestibular nucleus neuron, which was monosynaptically elicited by vestibular nerve stimulation. Atropine (25 nA) was continuously given during the period indicated by a solid underline.

Table 1. Relationship between effects of iontophoretic phencyclidine (50-120 nA) and acetylcholine (200 nA) on spontaneous firing of lateral vestibular nucleus neurons monosynaptically activated by vestibular nerve stimulation

<table>
<thead>
<tr>
<th>Acetylcholine</th>
<th>Phencyclidine</th>
<th>Total</th>
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<tr>
<td>↑</td>
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<tr>
<td>12</td>
<td>31</td>
<td>44</td>
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*Number of neurons. ↑: Increase, −: No effect, ↓: Decrease

ACh (Table 1).

Atropine at doses of 25–50 nA was iontophoretically applied for 3–5 min. Thirty seconds after the start of the application, PCP, ACh and glutamate were given sequentially for 30–40 sec. Under these conditions, atropine completely inhibited the increase in firing rate caused by PCP as well as that by ACh without affecting the glutamate-induced firing (Fig. 3). The PCP- and ACh-induced firing reappeared 1–2 min after the cessation of atropine application. Blockade by atropine (25–50 nA) of the PCP- and ACh-induced firing was observed in all 7 neurons examined.

Discussion

There is considerable evidence that ACh acts as a neurotransmitter in the primary afferent transmission in the vestibular nuclei. Choline acetyltransferase-containing nerve terminals (19) and acetylcholinesterase (20) have been found in the nuclei. Eserine, a cholinesterase inhibitor, reportedly increased the amplitude of the field potential upon vestibular nerve stimulation, whereas scopolamine, an anticholinergic drug, decreased the amplitude (21, 22). Furthermore, we (16) demonstrated that iontophoretically applied atropine blocked the spike generation upon vestibular nerve stimulation in the LVN monosynaptic neuron, of which spontaneous firing was increased by iontophoretic application of ACh. Atropine also blocked the ACh-induced firing without affecting glutamate-induced firing. The present data that about 40% of the LVN monosynaptic neurons were activated by ACh suggest that ACh acts as a neurotransmitter from the primary afferent fibers to some part of the LVN neurons.

In the LVN monosynaptic neurons in which the spike generation with vestibular nerve stimulation was blocked by atropine, ion-
trophoretically applied PCP increased the spontaneous firing in about 3/4 of the neurons, of which the firing was increased by ACh. The PCP-induced firing was also blocked by atropine, and a good correlation between the effects of PCP and ACh was observed. These results strongly suggest that PCP acts on the cholinergic receptor of the LVN neurons in a similar manner to ACh, although the possibility that the compound releases ACh from the nerve terminals could not be completely excluded. Actually, PCP reportedly displaced muscarinic antagonists such as quinuclidinyl benzilate from the muscarinic receptors in the brain (23, 24). The present results concerning the effects of PCP are in line with findings by Hyvarinen et al. (25) that neuronal activity in the posterior-parietal association cortex of the monkey was enhanced by intravenous and intramuscular administration of moderate doses of PCP. In contrast, inhibitory effects of PCP on the ACh-induced firing has been reported in the pyramidal cells of the rat hippocampus. However, the inhibitory effects were observed only at higher doses of PCP that might produce nonspecific local anesthetic action (26). In the present study, higher doses of PCP reduced the spike height in the LVN neuron without affecting the spike generation upon vestibular nerve stimulation, suggesting that the compound possesses local anesthetic activity. Therefore, it is considered that PCP is not so much a cholinergic antagonist as a cholinergic agonist that activates the LVN monosynaptic neurons receiving input from the vestibular nerve.

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References


