EFFECTS OF DIPHENIDOL ON THE CENTRAL VESTIBULAR AND VISUAL SYSTEMS OF CATS

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It is well known that anticholinergic agents such as atropine and scopolamine are effective as anti-motion sickness drugs, however, the mechanism of action in the vestibular system is inadequately understood. It has been demonstrated by several investigators (1–5) that neurons in the vestibular nucleus are excited by local application of acetylcholine. Gerebtzoff (6) has shown a histochemical localization of cholinesterase in the vestibular nucleus of cats. Contents of the enzyme were relatively high in the superior and lateral parts of the nucleus, but low in the medial and inferior parts. According to the hypothesis of Wood and Graybiel (7), motion sickness occurred when activation of the central cholinergic system disturbed the balance of vestibular reactions, and protection would be achieved by utilizing anticholinergic drugs.

Diphenidol (1,1-diphenyl-4-piperidine-1-butanol) which is related chemically to trihexyphenidil and chlorphenianol has been found effective in the treatment of motion sickness (8–11). Leonard et al. (12) have shown that diphenidol is equal in anti-emetic potency to chlorpromazine and that diphenidol has a greater selectivity of pharmacological action in experimental animals. The present experiments using cats were designed to study the effects of diphenidol on evoked responses in the central vestibular and visual systems.

METHODS

Fourteen adult cats of both sexes, weighing 2.5–4.5 kg, previously confirmed to be normal under gross behavioral experiments were used. All surgical procedures were performed under diethyl-ether anesthesia. After the trachea, femoral artery and radial vein had been cannulated, the left tympanic bulla was exposed under ventral approach. After trepanation of the inferior wall of the bulla, the mucous membrane in the middle ear was removed. A small concentric bipolar stainless steel electrode was inserted into the vestibular nerve through the round window according to the method of Fredrickson et al. (13). The head of the animal was fixed on a stereotaxic instrument of Todai-Noken type and the skull over the lateral vestibular nucleus was removed to allow insertion of the recording electrode.

For experiments of the visual pathway, the skull over the left lateral gyrus (visual cortex) was removed and small holes were made for insertion of stimulating and recording electrodes into the ipsilateral optic tract and lateral geniculate nucleus. Location of the electrode tips
was determined with the aid of a stereotaxic brain atlas of Snider and Niemer (14): the lateral vestibular nucleus (-6.5, 3.0, 2.5 to -4.0), optic tract (11.0, 7.0, -4.0) and lateral geniculate nucleus (5.0, 10.0, 4.0). A silver ball-tipped electrode was used for recording from the visual and motor cortices. Wound edges were sprayed with 8% lidocaine repeatedly throughout the experiments. After completion of all operative procedures, the animal was immobilized with gallamine triethiodide (5 mg/kg/hr i.v.) and sustained by artificial respiration after which it was placed in a room maintained at 28°C, and at least 3 hr allowed for recovery from diethyl-ether anesthesia before the recording was begun.

Evoked responses were amplified with a dual-beam oscilloscope (Nihon Kohden, VC-7) and then photographed using a long-recording camera. Spontaneous EEG in the motor cortex and hippocampus, heart rate and blood pressure were recorded using an ink-writing oscillograph (Nihon Kohden, WI-180-TR).

The vestibular nerve was stimulated every 5 sec with square waves, consisting of duration of 0.01 msec and intensity of 5 to 10 V, delivered from an electronic stimulator (Nihon Kohden, MSE-40). The optic tract was stimulated every 3.2 sec with square wave pulses, 0.05 msec in width and 12 V in intensity.

Diphenidol (Nippon Shinyaku Co.) dissolved in distilled water was injected into the radial vein.

At the end of each experiment, a 22.5 V direct current was applied to the electrodes so that the sites of the recording and stimulating electrodes could be confirmed histologically. One such site of the lateral vestibular nucleus is illustrated in middle panel of Fig. 1.

Statistical significance of the data was determined by Student's t-test.

RESULTS

1. Effects of diphenidol on evoked responses in the lateral vestibular nucleus

Fig. 1 shows an alteration in evoked responses to electrical stimulation of the vestibular nerve during the different placement of the electrode from a dorsal to ventral trajectory in the lateral vestibular nucleus. A typical response consisting of three negative waves (N1, N2 and N3) was elicited in the middle portion of the nucleus. The N3-wave was a small negative potential with short latency, and the N2-wave had the largest amplitude and sometimes two or three peaks. The N1-wave also had a small negative deflection following the N2.

Mean latencies of the N1, N2 and N3-waves in 7 animals were 0.50 ± 0.03 (S.E.), 1.09 ± 0.03 and 2.43 ± 0.21 msec respectively, and mean amplitudes of these waves were 96.7 ± 10.7 (S.E.), 176.3 ± 14.5 and 123.4 ± 17.5 μV respectively.

As shown in Fig. 2, amplitudes of the N1- and N3-waves were not affected by intravenous administration of diphenidol up to 0.5 mg/kg, however, the N2-wave was significantly (P<0.05) reduced in amplitude at a dose of 0.5 mg/kg. Two mg/kg of the drug produced a marked decrease in amplitudes of the N1 and N3-waves, and these waves completely disappeared after 16 mg/kg though N1-wave was still observed in the highest dose. Fig. 3 illustrates a typical example of the effects of diphenidol on the evoked responses recorded from the lateral vestibular nucleus.
FIG. 1. Evoked responses to vestibular nerve stimulation during different placements (1 to 10) of the electrode from a dorsal to ventral direction in the lateral vestibular nucleus. Abbreviation: Stim: stimulation; N₁, N₂ and N₃: the first, second and third negative waves.

FIG. 2. Effects of diphenidol on the amplitude of N₁, N₂ and N₃ waves of evoked responses in the lateral vestibular nucleus induced by vestibular nerve stimulation. The mean % change (n=7) in each wave is shown following increasing doses of diphenidol (i.v.). Vertical lines represent the standard error. *: p<0.05.
Fig. 3. An example of effects of diphenidol on the evoked response in the lateral vestibular nucleus induced by vestibular nerve stimulation. A: Before, B: 5 min after 1 mg/kg (i.v.) of diphenidol, and C: 5 min after 4 mg/kg. Calibration: 1 msec, 100 μV.

Fig. 4. Time-course of % change in the N₁, N₂, and N₃-waves of evoked responses in the lateral vestibular nucleus induced by vestibular nerve stimulation after intravenous injection of 0.5 mg/kg of diphenidol. Vertical lines represent the standard error.
Fig. 4 shows a time-course of change in the N₁, N₂, and N₃-waves after administration of diphenidol in a dose of 0.5 mg/kg. Amplitudes of the N₁ and N₂-waves slightly increased 15 min after the injection, and then gradually recovered to the respective control levels. Amplitude of the N₃-wave, however, was markedly reduced 5 min after the administration and recovered to the control level at 90 min.

2. Effects of diphenidol on evoked responses in the lateral geniculate nucleus and visual cortex

Evoked potentials of the lateral geniculate nucleus in response to electrical stimulation of the optic tract consisted of tract and radiation waves. Evoked responses to the optic tract stimulation in the visual cortex had four positive waves (P₁ to P₄) and a long-lasting negative (N) wave following P₁.

![Image of Fig. 5](image1.png)

![Image of Fig. 6](image2.png)
Effects of diphenidol on the spontaneous EEG recorded from the motor cortex (MC), visual cortex (VC) and hippocampus (HPC). Time scale: 1 sec.

As shown in Fig. 5, the radiation wave was markedly depressed by administration of 1 mg/kg of diphenidol, without affecting the tract wave. The decrease in amplitude of the radiation wave was dose-dependent and amplitudes at dose of 4 mg/kg were approx. 40% of the control. Latency of the evoked potentials, however, did not change by drug administration.

Fig. 6 demonstrates the effects of diphenidol on evoked responses of the visual cortex induced by optic tract stimulation. One mg/kg of diphenidol resulted in a slight decrease in amplitude of the P4-wave. At doses above 4 mg/kg, P4-wave was markedly depressed and P1 and P2-waves also showed a decreasing tendency in amplitude. Latency of the each wave, however, was not affected by the doses of diphenidol.

3. Effects of diphenidol on spontaneous EEG

Spontaneous EEG recorded from the motor and visual cortices and the hippocampus were not apparently modified by intravenous injection of diphenidol up to 4 mg/kg. At doses above 8 mg/kg, the cortical and hippocampal EEG altered into the high-voltage waves mixed with spindle bursts (Fig. 7). This resting pattern continued for 1 or 2 hr. and thereafter the EEG recovered to normal level.
4. Effects of diphenidol on blood pressure and heart rate

Maximum and minimum blood pressures recorded from the femoral artery were in the range of 110-150 and 70-110 mmHg respectively (N = 4). As shown in Fig. 8, diphenidol in doses of 1 to 4 mg/kg failed to produce significant effects on the blood pressure and heart rate, though slight undulation of the blood pressure was observed after drug administration. Slight decrease of the blood pressure and heart rate was found immediately after 8 mg/kg, and a marked but transient fall of the blood pressure was recorded after 16 mg/kg of diphenidol.

![Fig. 8. Effects of diphenidol on the heart rate and blood pressure (BP) as recorded from the femoral artery. Each large scale on the section paper corresponds to 1.5 min.](image)

DISCUSSION

Mickle and Ades (15) have firstly reported irregular responses of 1.0 to 1.25 msec latency from the lateral vestibular nucleus induced by electrical stimulation of ipsilateral vestibular nerve. Gernandt et al. (16) have also shown evoked responses in the vestibular nucleus with a latency of less than 1.0 msec. Subsequently, Shimazu and Precht (17) have demonstrated the field potentials in the vestibular nucleus elicited by vestibular nerve stimulation, which consist of initial positive to negative wave (P) and two negative waves (N_1 and N_2). Latencies of the P, N_1 and N_2-waves were 0.66, 1.06 and 2.40 msec respectively. The P-wave was interpreted as indicating the afferent presynaptic component, and the N_1 and N_2-waves were attributed to postsynaptic components which responded to nerve stimulation mono- and polysynaptically, respectively. In the present experiments, three negative potentials (N_1, N_2 and N_3-waves) were recorded from the lateral vestibular nucleus in response to ipsilateral vestibular nerve stimulation. In view of the latencies, these three waves were considered to correspond to P, N_1 and N_2-waves, respectively, which were described by Shimazu and Precht (17, 18).

In the present study, relatively small doses of diphenidol reduced the amplitude of N_2-wave without affecting the N_1 and N_3-waves. Larger doses of the drug produced a marked decrease in amplitude of the N_1 and N_2-waves, but the N_2-wave was not significantly depressed even at the highest dose. In the previous study (5), the N_3-wave, monosynaptically evoked component, was markedly enhanced by physostigmine and depressed by scopolamine; however, the N_1-wave was not altered by either the cholinergic agonist or antagonist. Feldberg and Vogt (19) have reported that the content of acetylcholine in
the vestibular nucleus is higher than that of the vestibular nerve. Therefore, it is assumed that diphenidol blocks the synaptic transmission in the lateral vestibular nucleus, in which the rate of acetylcholine synthesis and content of cholinesterase was relatively high.

As already reported by several investigators (20-22), the tract and radiation waves of evoked responses in the lateral geniculate nucleus induced by optic nerve stimulation were regarded as pre- and postsynaptic components respectively. It has been explained that the P1-wave of evoked responses in the visual cortex represents presynaptic potentials, while the P2 and N-waves postsynaptic ones.

The postsynaptic components of the lateral geniculate nucleus in response to electrical stimulation of the optic tract was markedly depressed by the dose of diphenidol, which did not affect the spontaneous EEG and blood pressure. Curtis and Davis (23) and Phillis et al. (24) have reported that the greater part of the lateral geniculate nucleus is excited by iontophoretical application of acetylcholine. Matsuoka and Domino (25) have demonstrated that most of the lateral geniculate neurons are excited following administration of physostigmine and the enhanced response induced herein is antagonized by scopolamine. The present results also suggest that diphenidol at relatively small doses may act as anticholinergic drug on the vestibular and visual systems.

SUMMARY

Central action of diphenidol was studied in gallamine-immobilized cats. Diphenidol markedly depressed the postsynaptic component of evoked responses in the lateral vestibular nucleus induced by electrical stimulation of the ipsilateral vestibular nerve without affecting the presynaptic component. In the visual system, diphenidol reduced the amplitude of the postsynaptic component, but not that of the presynaptic, regarding the lateral geniculate nucleus potentials in response to optic tract stimulation. The evoked responses in the visual cortex induced by optic tract stimulation were depressed by the drug. On the other hand, the cortical and hippocampal spontaneous EEG, blood pressure and heart rate were not apparently influenced by diphenidol in the dose which affected the evoked responses in the vestibular and visual systems. Anticholinergic properties of diphenidol on the central nervous system were discussed.

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