Effects of Afloqualone on Vestibular Nystagmus and the Lateral Vestibular Nucleus

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Abstract—To clarify the antivertiginous effect of afloqualone, an antispastic drug, we examined its action on the vestibular nervous system in cats. The results suggest that afloqualone inhibits vestibular nystagmus probably due to both inhibition of selective polysynaptic transmission and enhancement of the effects of GABA and glycine in the lateral vestibular nucleus (LVN), and its GABA-enhancing effect is thought to be attributable to the increased sensitivity of GABA receptors of the LVN neuron site.

The onset of vertigo is attributed to rapid failure of the integration of the motor and sensory systems controlled by the vestibular nervous system (1); motor system disturbance is likely to cause vertigo. Thus, it is important to examine whether antispastic drugs that act on the motor system to improve their disorders have an antivertiginous effect.

To examine whether afloqualone (2, 3), an antispastic drug, has an antivertiginous effect, we compared its effect with those of eperisone (4) in the same antispastic class and diphenidol (5), an antivertiginous drug, on vestibular nystagmus as an experimental model for vertigo. In addition, we evaluated the effect of afloqualone on the lateral vestibular nucleus (LVN) by an electrophysiological technique to clarify the mechanism of its action.

Cats (both sexes, 1.6–3.8 kg) prepared with spinal transection (L1) were fixed in a stereotaxic instrument (Institute of Brain Research, Faculty of Medicine, University of Tokyo) under ether and light ketamine anesthesia. All wound surfaces and compression sites were locally anesthetized with 0.01% dibucaine.

Vestibular nystagmus was induced in animals with blind-folded eyes by 40-second clockwise horizontal rotations at 30 rpm (an equiangular rate of 180°/sec) at intervals of 30 min and recorded as electronystagmograms (ENG) on a polygraph through a medical telemeter system (Nihon Kohden, ZB-114 and RZ-5, time constant: 0.1 sec). The habituation to rotatory stimulation was observed.

Table 1 shows the results obtained 60 min after oral administration of the test drugs. Afloqualone inhibited vestibular nystagmus dose-dependently at 5 mg/kg or more. In contrast, eperisone had little inhibitory effect on nystagmus at 100 mg/kg, inhibiting the cat spinal reflex (4), but prolonged its duration and increased its amplitude. Intravenous injection of 20 mg/kg of eperisone, however, inhibited all parameters. Diphenidol exerted little effect on nystagmus at 20 mg/kg; however, it did inhibit it at 50 mg/kg, although at this dose, diphenidol induced marked mydriasis probably due to its anticholinergic action.

A previous report shows that eperisone administered by microiontophoresis inhibits synaptic transmission in the cat vestibular nuclei (6), but it remains unknown why this drug acts differently depending on the route of administration when vestibular nystagmus is used as a parameter.

Since afloqualone inhibited vestibular nystagmus, we attempted to clarify the mechanism of its action through evaluation of its effects on the vestibular nerve-LVN and cerebellum-LVN routes (1, 7) among the
Table 1. Effects of afloqualone, eperisone and diphenidol on vestibular nystagmus in cats

| Dose mg/kg | Route | n  | Parameters of nystagmus (%*, mean±S.E.) |  |  |  |  |  |  |
|-----------|-------|----|----------------------------------------|---|---|---|---|---|---|
|           |       |    | Frequency                               |  |  |  |  |  |  |
|           |       |    | Per. b                                  |  |  |  |  |  |  |
| Control   | —     | 5  | 63.6±9.45                               | 66.3±10.71 | 68.5±9.47 | 54.9±5.43 | 65.0±8.90 | 62.8±3.19 |
| Afloqualone| 2     | 4  | 55.6±4.07                               | 72.7±11.73 | 76.2±10.36 | 102.4±17.87 | 74.0±15.71 | 75.6±7.18 |
|           | 5     | 5  | 32.3±5.84                               | 57.3±24.71 | 57.1±7.47  | 85.0±9.31  | 46.3±15.51 | 31.2±15.51 |
|           | 10    | 5  | 4.2±4.22                                | 9.3±6.53   | 8.4±8.34   | 10.3±6.34  | 14.0±14.0  | 17.9±7.40  |
| Eperisone | 100   | 4  | 51.1±9.97                               | 56.3±10.30 | 119.4±35.84 | 180.5±65.06 | 92.8±11.01 | 125.9±18.32 |
| Diphenidol| 20    | 4  | 59.5±11.81                              | 67.1±14.96 | 67.1±12.16 | 71.6±10.43 | 71.7±6.73  | 69.8±7.42  |
|           | 50    | 2  | 9.5±6.85                                | 6.5±7.34   | 14.0±9.0   | 9.0±5.73   | 8.5±10.0   | 11.0±5.59  |

(Marked mydriasis was observed)

Control — i.v. 5 62.7±4.79 65.4±4.44 79.8±8.59 71.9±8.62 66.7±13.46 70.1±13.46
Eperisone 20 i.v. 6 32.0±6.85 41.3±7.34 51.3±9.0 48.4±5.11 47.1±9.19 57.1±5.59

*The rate (%) of each parameter 60 min after an oral dose or 15 min after an intravenous dose of each test drug against the value 60 min before administration.

bPer-rotatory nystagmus. cPost-rotatory nystagmus. *P<0.05, **P<0.01 (Kruscal Wallis test).
major routes of projection to the LVN by an electrophysiological technique.

In the following experiments, cats were anaesthetized with α-chloralose, immobilized with gallamine and maintained under artificial respiration. A bipolar stimulating electrode was inserted into the vestibular nerve through the inter acoustic meatus. For recording, we used a glass-insulated tungsten microelectrode (the tip was 5 μm in diameter and 20 μm in length). A 4-barreled microiontophoretic pipet was attached to each electrode, which was inserted into the left LVN [P: 8.0, L: 4.0, H: −2 to −3.5 (8)]. Data were processed by a medical data processor (Nihon Kohden, ATAC-450).

Spikes on the monosynaptic N₁ wave (N₁ spike) and polysynaptic N₂ wave (N₂ spike) obtained from an extracellular single neuron (9, 10) in response to stimulation of the vestibular nerve (1/15 Hz, 0.05 msec, square wave pulse) were displayed in post-stimulus latency histograms in bird’s eye view indicating the results of intravenous injection of afloqualone at 2–10 mg/kg (7–9 cases, Fig. 1a).

Afloqualone had no significant effect on the N₁ spike generation and spike latency, but significantly inhibited the N₂ spike generation at 0.25 to 6 min after injection of 5 and 10 mg/kg of the drug.

Acetylcholine is considered to be the transmitter controlling monosynaptic transmission between the vestibular nerve and LVN, and diphenidol is considered to exert the antivertiginous effect through inhibition of monosynaptic transmission by its anticholinergic action (1, 5, 11). Afloqualone had no effect on the N₁ spike or central cholinergic system (12), suggesting that its antivertiginous effect is derived from other than the cholinergic system.

Then, we examined the interaction in the LVN between GABA (7) or glycine (13), an inhibitory transmitter in the LVN, and afloqualone.

At doses of 0.57–1 mg/kg, i.v., afloqualone enhanced the inhibitory effects of GABA (0.5 M, 5–40 nA) and glycine (0.5 M, 15–40 nA) by 18.6–120.7% in 7 of 8 animals and 19.2–89.2% in all 5, respectively (Fig. 1b).

To clarify whether or not the GABA-enhancing action of afloqualone originates in cerebellar Purkinje neurons (7) that project...
GABAergic neurons to the LVN, we evaluated the effect of this antispastic drug on the cerebellar inhibition (14), namely, the inhibition of spontaneous firing of LVN neurons due to stimulation (0.5 Hz, 0.05 msec, square wave pulse) of the cell body layer (300-600 µm from the surface) of Purkinje cells in the cerebellar vermis.

At a dose of 1 mg/kg, i.v., afloqualone significantly enhanced cerebellar inhibition in all 4 animals. The spike latencies of pre- and posttreatment were 16.7±2.26 msec and 21.6±2.38 msec, respectively (mean±S.E., P<0.01, paired t-test). Afloqualone also had the following effects: further enhancement of the effect of GABA (0.5 M, 20 and 25 nA, spike latency: 19.9±4.32 msec and 27.3±4.75 msec, P<0.05) and inhibition of the effect of bicuculline (0.005 M, 40 and 50 nA, spike latency: 9.3±3.04 msec and 15.5±2.66 msec, P<0.05) (Fig. 1c).

These findings indicate that the enhanced effect of GABA by afloqualone in the LVN was the result of enhancement of the effect of GABA from Purkinje neurons.

To determine whether the GABA-enhancing effect was of presynaptic origin (an increased GABA release) or of postsynaptic origin (an increased sensitivity of GABA receptors), we compared the effect of afloqualone on the spontaneous firing rates in the LVN and Purkinje (cerebellar vermis) neurons. After the termination of experiments, the recording and stimulating sites were histologically verified using hematoxylin-eosin stain.

Afloqualone inhibited spontaneous firing in Purkinje neurons at 1 mg/kg, i.v., in 5 of 6 animals (20.1-36.8%) and in LVN neurons at 0.57-1 mg/kg, i.v., in 9 of 10 (24.7-45.2%). Such inhibitions were transient in 4 and 7 animals, respectively. The time required for recovery to 85% or more was 2.0±0.5 min and 10.5±1.98 min (mean±S.E., P<0.01, Cochran’s t-test), respectively. Afloqualone exerted the definitely stronger inhibitory effect on the latter (Fig. 1b shows an example of LVN).

The evidence that afloqualone inhibited spontaneous firing of neurons both in Purkinje cells and the LVN, the latter more potently, indicates that its GABA-enhancing effect in the LVN was due to an increased sensitivity of GABA receptors in the postsynaptic region, i.e., the LVN neuron site, rather than an increased release of GABA of presynaptic origin.

The brainstem reticular formation-LVN route is another major route of projection to the LVN (1), but afloqualone with no anesthetic or hypnotic property is unlikely to exert an inhibitory effect on this route (15).

The above findings suggest that afloqualone inhibits vestibular nystagmus probably through both inhibition of selective polysynaptic transmission in the vestibular nerve-LVN route and enhancement of the effects of GABA and glycine in the LVN, and that its enhancing effect on GABA is attributable to the increased sensitivity of GABA receptors of the LVN neuron site in the cerebellum-LVN route.

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