CHARACTERISTICS OF THE GABA-POTENTIATING ACTION OF HARMAN

I. V. Komissarov and I. I. Abramets

UDC 615.214.22.015.42:[612.822.1:612.398.192:547.466.3

KEY WORDS: harman; GABA-ergic synapses; spinal cord.

Harman [3, 13], norharman [3, 8], and β-carboline-3-carboxylic acid [6] interact with high affinity with benzodiazepine (BD) receptors in the cytoplasmic membranes of the brain, displacing labeled BD from their specific binding sites. These β-carbolines are regarded as possible endogenous ligands of benzodiazepine receptors [3, 5, 12, 13]. However, unlike BD, which potentiate pre- and postsynaptic inhibition in synapses of the spinal cord [7, 9], β-carbolines facilitate spinal reflexes and have an excitatory (convulsant) effect in behavioral experiments on animals [4, 10, 11]. The excitatory effects of the β-carbolines may also be the result of their multisynaptic action [4].

Benzodiazepine tranquilizers potentiate effects of GABA on primary afferents in nerve tissue culture [6] and in the isolated spinal cord of amphibians [9] and rats. The investigation described below showed that harman has a similar effect, and it analyzed the GABA-potentiating action of harman.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated perfused spinal cord of young rats aged 9-15 days. Full details of the method were described previously [1].

The effect of harman (hydrochloride, 1×10^{-5} to 1×10^{-4} M) on evoked mono- and polysynaptic ventral root potentials and on the dorsal root potential evoked by stimulation of the dorsal root of the neighboring segment (DR-DRP) were investigated. All evoked potentials were excited by stimulation of dorsal root L3 by square pulses of current (0.3 msec, 0.1 Hz, 2-8 thresholds) and recorded in ventral roots L3 (monosynaptic) and L4 (polysynaptic) or in dorsal root L4 (DR-DRP).

The characteristic action of harman alone (1×10^{-8} to 1×10^{-4} M) and its effect, with an exposure of 15 min, on GABA (1×10^{-5} to 1×10^{-4} M)-induced primary afferent depolarization or hyperpolarization of motoneurons were studied by recording electrotonic potentials of the dorsal or ventral roots (L3). Synaptic transmission in the spinal cord was blocked by perfusing it with a solution deficient in Ca^{++} ions (0.2 mM) and containing an excess (10 mM) of Mg^{++} ions [1, 2].

Dependence of the GABA-potentiating action of harman (1×10^{-5} M) on the concentration of chloride ions in the solution used to perfuse the spinal cord was studied. The initial chloride concentration (124 mM) was increased to 165 mM by the addition of 41 mM choline chloride to the solution or it was reduced to 83 mM by replacing sodium chloride by the equivalent amount of sodium sulfate (pH of the solutions 7.4-7.6).

In a separate series of experiments the effect of harman (1×10^{-5} to 1×10^{-4} M) on the action potential was studied in a fine bundle of rat sciatic nerve fibers.

Each version of the experiments was carried out on 4-6 preparations of the spinal cord. The results were subjected to statistical analysis in the usual way.

EXPERIMENTAL RESULTS

During perfusion of the isolated spinal cord with solution containing harman (1×10^{-5} to 1×10^{-4}) an increase in the amplitude and duration of the dorsal root potential (DR-DRP) due to electrical stimulation of the dorsal root of the neighboring segment was observed (Fig. 1C). A similar change in DR-DRP likewise was ob-

Department of Pharmacology, A. M. Gor'kii Donetsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 10, pp. 61-63, October, 1982. Original article submitted April 21, 1982.

 0007-4888/82/9410-1382$07.50

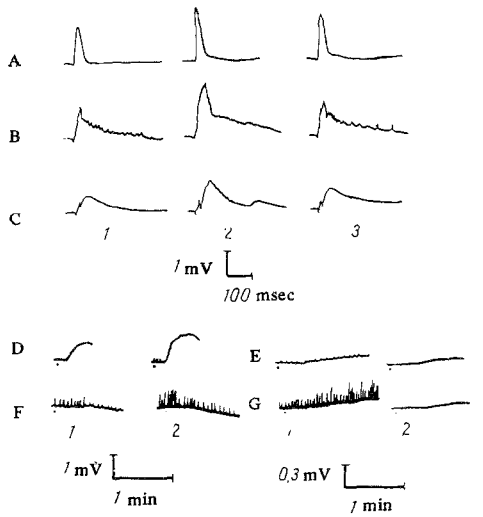

Fig. 1. Effect of harman on evoked potentials of ventral and dorsal roots of isolated rat spinal cord. A-C) potentials evoked in ventral root L3, ventral root L4, and dorsal root L4 respectively by electrical stimulation of dorsal root L3: 1) before perfusion, 2) after perfusion of spinal cord for 15 min with solution containing harman (1×10^{-5} M), 3) 15 min after rinsing; D-F) electrotonic potentials arising in dorsal and ventral root respectively during perfusion of spinal cord with GABA (1×10^{-4} M) solution: 1) before exposure to harman, 2) after preliminary exposure to harman (1×10^{-5} M); E, G) changes in polarization of dorsal and ventral roots respectively under the influence of harman during perfusion of spinal cord: 1) with ordinary salt solution, 2) with solution containing an excess of Mg^{++} ions and a deficiency of Ca^{++} ions.

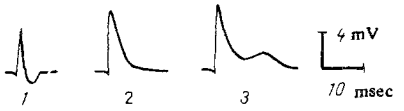

Fig. 2. Effect of harman on action potential in bundle of rat sciatic nerve fibers: 1) before, 2, 3) 10 and 20 min respectively after action of harman in concentration of 1×10^{-4} M.

served under the influence of BD and was interpreted as the result of potentiation of presynaptic inhibition of GABA-ergic nature of primary spinal cord afferents [7]. In fact, the primary afferent depolarization due to GABA (1×10^{-4} M) was significantly strengthened if the spinal cord was perfused beforehand for 15 min with a solution containing 1×10^{-5} M harman (Fig. 1D).

However, harman also potentiated orthodromically induced mono- and polysynaptic ventral root potentials (Fig. 1A, B), which could not be connected with the GABA-potentiating effect of harman, for this substance potentiates GABA-induced hyperpolarization of motoneurons (Fig. 1F). Potentiation of the ventral root potentials and, to some extent the potentiation of DR-DRP may evidently be the result of an action of harman that is independent of its effect on GABA-ergic systems of neurons. This view is confirmed by the fact that harman has a true depolarizing effect on primary afferents and motoneurons (Fig. 1E, G). This action is due to the direct effect of harman on afferent terminals and motoneurons, for its persists when interneuron function is blocked by perfusion of the spinal cord with a solution containing an excess of Mg^{++} and a deficiency of Ca^{++} (Fig. 1).

The ability of harman to potentiate evoked potentials in the ventral and dorsal roots is analogous to the effect of tetraethylammonium and 4-aminopyridine, which facilitate conduction in synapses of the rat spinal

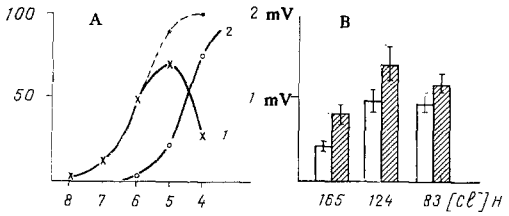

Fig. 3. Dependence of GABA-potentiating action on concentrations of harman and chlorides in medium. A) Dependence of GABA-potentiating (1) and depolarizing (2) effects of harman during recording of electrotonic dorsal root potentials. Abscissa, harman concentration (in M); ordinate, effect (in percent of maximal). Broken line shows interpolation of initial segment of curve 1 with Hill's index = 1. To obtain curve 1 GABA was used in a concentration of 1×10^{-4} M. B) Responses of primary afferents induced by GABA (1×10^{-4} M) in absence (unshaded columns) and presence (shaded columns) of harman (1×10^{-6} M) in medium with different chloride ion concentrations.

cord [2] as a result of depression of potassium conductance of the membranes of nerve cells and increased excitability of the latter [14]. Experiments on an isolated bundle of fibers from the rat sciatic nerve showed that the descending phase of the action potential is lengthened and its amplitude somewhat increased in the presence of harman in concentrations above 1×10^{-5} M (Fig. 2). It can be tentatively suggested that this effect was due to the action of harman on the permeability of nerve fiber membranes for K^+ ions, and the same effect of harman on primary afferent terminals lies at the basis of its characteristic depolarizing action on these structures.

The characteristic depolarizing effect of harman on primary afferents was exhibited at concentrations over 1×10^{-6} M. It developed parallel to the decrease in the GABA-potentiating action of harman (Fig. 3A) and, judging from the ratio between the effective concentrations, it was the cause of the reduction in the GABA-potentiating effect when the harman concentration was increased.

The GABA-potentiating effect of harman depended essentially on the concentration of chloride ions in the medium: in chloride concentrations of 165, 124, and 83 mM the increase in the depolarizing effect of GABA under the influence of harman (1×10^{-6} M) was 100, 46, and 29% respectively (Fig. 2B). This is evidence that the GABA-potentiating effect of harman, like the analogous effect of chlordiazepoxide, is due to the influence of GABA on chloride ionophores coupled with GABA receptors in the membranes of primary afferent terminals.

Like BD [6, 9], harman thus has a GABA-potentiating effect due to its primary action on chloride ionophores coupled with GABA receptors. If the concentrations of harman are high enough this effect is neutralized by the increased excitability of the neuron membranes, evidently as a result of inhibition of the potassium conductance of nerve cell membranes by harman.

LITERATURE CITED

1. I. I. Abramets, N. A. Kozlova, and I. V. Komissarov, Fiziol. Zh. SSSR, No. 8, 1160 (1981).
2. Z. A. Tamarova, A. I. Shapovalov, and B. I. Shiryaev, Neirofiziologiya, No. 5, 530 (1978).
3. M. M. Airaksinen and E. Mikkonen, Med. Biol., 58, 341 (1980).
4. H. O. Borbe, U. Wollert, W. E. Müller, et al., Pharmacol. Biochem. Behav., 14, 693 (1981).
5. C. Braestrup, M. Nielsen, and C. E. Olsen, Proc. Natl. Acad. Sci. USA, 77, 2288 (1980).
6. D. W. Choi, D. H. Farb, and L. D. Fischbach, Nature, 269, 342 (1977).
7. D. R. Curtis, C. J. A. Game, and D. Lodge, Br. J. Pharmacol., 56, 307 (1976).
8. A. M. Morin, I. A. Tanaka, and C. G. Wasterlain, Life Sci., 28, 2257 (1981).
9. A. Nistri and A. Costandi, Neuropharmacology, 17, 127 (1978).
10. R. A. O'Brien, W. Schlosser, N. M. Spirt, et al., Life Sci., 29, 75 (1981).
11. P. Polc, N. Ropert, and D. M. Wright, Brain Res., 217, 216 (1981).
12. H. Rommelspacher, Pharmakopsychiatria, 14, 117 (1981).
13. H. Rommelspacher, C. Nanz, H. O. Borbe, et al., Naunyn-Schmiedeberg's Arch. Pharmakol., 314, 97 (1980).
14. A. I. Shapovalov and B. I. Shiryaev, (Shiriaev), Exp. Brain Res., 32, 180 (1978).