EFFECT OF AMINO ACID DERIVATIVES OF β-CARBOLINE ON EVOKED ACTIVITY OF HIPPOCAMPAL NEURONS

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UDC 612.825.266.014.2/.014.46:615.214.22/.08

KEY WORDS: hippocampal slices; anxiogenic β-carbolines; reciprocal inhibition.

The attention of research workers studying the molecular mechanisms of formation of anxiety states has recently been drawn to β-carboline derivatives. It has been shown that several β-carboline-3-carboxylates possess marked anxiogenic properties and that their pharmacological activity is mediated through benzodiazepine receptors (BDR) [4]. The existence of endogenous anxiogenic β-carboline derivatives is considered to be likely [5].

It was shown previously that a physiological model of surviving hippocampal slices adequately reflects the specific features of interaction of BDR with agonists, antagonists, and reverse agonists of benzodiazepines (BD) and, in conjunction with biochemical data, it can be used to determine the direction of the pharmacological activity of BDR ligands [3].

In the investigation described below the action of synthetic amino acid derivatives of β-carboline-3-carboxylate on this experimental model was evaluated in order to detect the probable anxiogenic activity of these compounds.

EXPERIMENTAL METHOD

Experiments were carried out on surviving transverse hippocampal slices of Wistar rats by the method described previously [1]. Unit activity was recorded in area CA1 during stimulation of Schaffer's collaterals. The principal indicator of integral unit activity during extracellular recording was the amplitude of the population spike (PS), the negative potential reflecting the combined discharge of the pyramidal neurons of that particular area. During the testing of inhibition of paired pulses, its measure was the ratio Ats·100%/Acs, where Ats is the amplitude of the testing spike (TS; the response to the second stimulus in the pair), Acs the amplitude of the conditioning spike (CS; the response to the first stimulus). The methylamide of β-carboline-3-carboxylate (MA) was synthesized by the method described previously [2]. Methyl esters of glycinamides and leucinamides of β-carboline-3-carboxylate were obtained from β-carboline-3-carboxylate and the methyl esters of glycine and leucine by the carbodiimide method. The compounds were dissolved in ethanol up to a concentration of 20 mM and, immediately before application, they were diluted with 0.14 M NaCl to a working concentration of 5 μM. In the control, the corresponding quantity of ethanol was added to the perfusion fluid. Activity of the compounds was compared on the same preparation in each experiment.

EXPERIMENTAL RESULTS

Activity of MA, a reverse BD agonist, capable of inducing an anxiety state in man and animals, was studied by the use of three types of stimulation. During stimulation by single pulses with a frequency of 0.1 Hz application of MA (5 μM for 15 min) led to facilitation of evoked activity, which was expressed as the appearance of additional PS (although they were absent in the control), and also as an increase in amplitude of the basic PS by 5-25% (24 experiments; Fig. 1b). During high-frequency stimulation with series of 10-20 pulses with a frequency of 1 Hz, during application of MA generation of additional spikes was observed in all cases. In the control, under analogous conditions, their stimulation was not observed (nine experiments). Activity of MA during paired stimulation took the form of predominant facilitation of TS. In a series of 14 experiments, the increase in TS was 105-430%, whereas the increase in CS was 7-22% (Fig. 2).

Laboratory of Functional Synaptology, Brain Institute, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 11, pp. 563-565, November, 1988. Original article submitted May 26, 1987.

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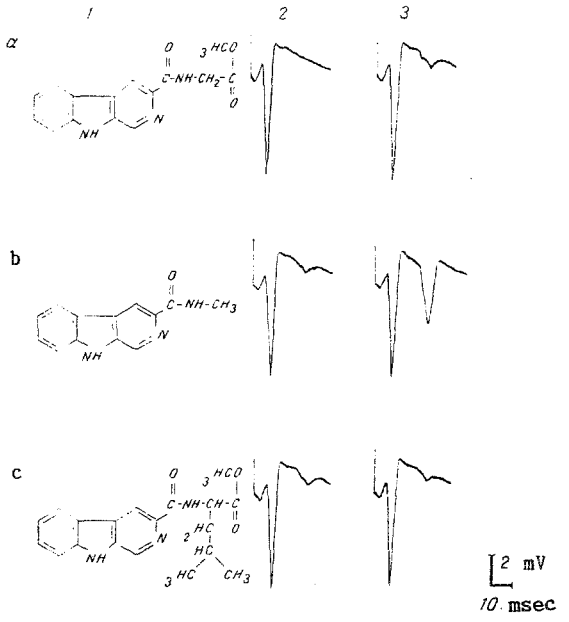

Fig. 1

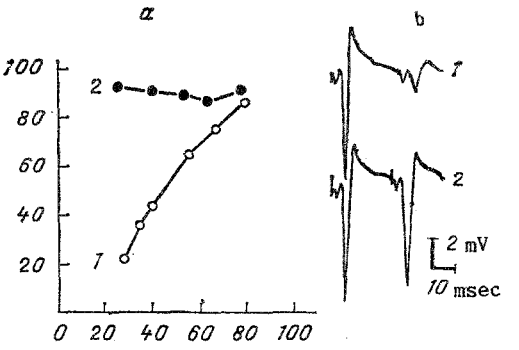

Fig. 2

Fig. 1. Action of β-carboline derivatives on evoked potential during stimulation by single pulses. 1) Formula of compound, 2) control, 3) after application of compound (5 μM for 15 min). a) Effect of LA, b) effect of MA, c) effect of LA.

Fig. 2. Action of MA on evoked potential during paired stimulation. a) Graph showing dependence of ratio Ats·100%/Acs on interval between pulses. Abscissa, interval between pulses (in msec); ordinate, Ats·100%/Acs; b) traces of responses from this particular experiment. 1) Control, 2) after application of compound (5 μM, for 15 min).

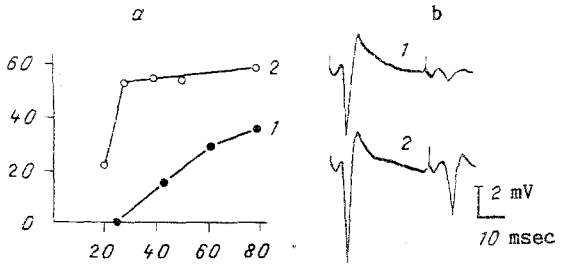

Fig. 3. Action of GA on evoked potential during paired stimulation. Legend as to Fig. 2.

Activity of the methyl ester of β-carboline-3-carboxylate glycinamide (GA) also was studied by the use of three types of stimulation. During stimulation with a frequency of 0.1 Hz application of the substance (5 μM for 15 min) caused an increase in the amplitude of PS by 3-10% and the appearance of additional PS in two of the six experiments. Traces of responses from an experiment in which application of GA caused the appearance of an additional PS are shown in Fig. 1a. Stimulation with a frequency of 1 Hz revealed more clearly the presence of additional PS, arising during application of the compound. In this case additional PS were observed in all six of the above experiments. During paired stimulation (seven experiments) predominant facilitation of TS took place after application of GA. This effect was weaker than in the case of MA. Complete abolition of depression of TS was not observed in any of the experiments (Fig. 3).

The methyl ester of β-carboline-3-carboxylate leucinamide (LA) did not exhibit any marked or unambiguous activity in this particular test system (five experiments; Fig. 1c).

Identification of the group of BDR ligands with anxiogenic and proconvulsant activity (reverse agonists of BD) has broadened our ideas on the spectrum of effects mediated by these

receptors. The action of MA, a representative of this group belonging to the β-carboline family, on evoked activity of hippocampal neurons was opposite to the effects of BD which, with respect to this model, have a potentiating effect on depression of TS, and suppress seizure activity [7]. The results suggest that under the influence of MA and GA, GABA-ergic reciprocal inhibition in hippocampal slices is suppressed. With respect to their ability to inhibit specific binding of labeled flunitrazepam, the compounds tested can be arranged in the following order: MA > GA > LA [6]. It follows from the results described above that these compounds are arranged in the same order with respect to their ability to induce seizure discharges and to suppress inhibition. Correlation is thus observed between the affinity of β-carboline derivatives for BDR and their electrophysiological activity. This similarity of the electrophysiological effects of MA and GA permits the suggestion to be made that GA also possesses anxiogenic activity in vivo; LA evidently has no anxiogenic properties.

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EFFECT OF 1,4-BENZODIAZEPINE TRANQUILIZERS ON BRAIN XANTHINE OXIDASE ACTIVITY IN RATS

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UDC 615.214.22:547.891.2/.015.4:616.831-008.931:577.152.1/.076.9

KEY WORDS: tranquilizers; 1,4-benzodiazepine; xanthine oxidase; rat brain.

Much interest has been aroused in recent years by the study of the molecular mechanisms of action of tranquilizers of the 1,4-benzodiazepine series, which have found extensive applications in clinical practice. An important role in the realization of the effect of benzodiazepines is played by purinergic mechanisms [5]. For instance, competitive relations have been found between benzodiazepines and adenosine for receptor binding sites in synaptosomes [4] and adenosine uptake is inhibited by benzodiazepines [5].

Skolnick and co-workers postulated that "endogenous benzodiazepines" of purine nature, especially products of adenosine-5'-monophosphate and adenosine metabolism, namely inosinic acid, inosine, and hypoxanthine, may be formed in nerve cells; these products may evidently function as endogenous ligands of benzodiazepine receptors [6].

The present writers [1, 2] have shown that administration of tranquilizers of the 1,4-benzodiazepine series inhibits activity of membrane-bound 5'-nucleotidase in brain tissue. It was also found that 1,4-benzodiazepines induce activity of cytosol enzymes — AMP deaminase and adenosine deaminase — to a considerable degree, so that the concentrations of inosinic acid and inosine in the brain cells increase after administration of tranquilizers.

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(Presented by Academician of the Academy of Medical Sciences of the USSR T. T. Berezov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 11, pp. 565-568, November, 1988. Original article submitted January 14, 1988.

0007-4888/88/0011-1572$12.50