ROLE OF CHOLINERGIC MECHANISMS IN THE MECHANISM OF ACTION OF BICUCULLINE

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UDC 615.217.3.015.45:612.826.2

The hypothesis of a possible role of cholinergic structures in the mechanism of the action of bicuculline was tested by screening and electrophysiological methods. Neither muscarinic (M-) nor nicotinic (N-) cholinolytics (benactyzine, atropine, aprophen,* and pediphen†) abolished convulsions produced in mice by bicuculline. Meanwhile substances inducing the accumulation of γ-aminobutyric acid (GABA) in the brain, such as aminohydroxyacetic acid (AHAA) and depakine, had a well marked protective action against bicuculline convulsions. The electrophysiological experiments also showed that the M-cholinolytic benactyzine does not abolish the effects of bicuculline. Bicuculline was shown to reduce the depression of the test response in the recovery cycle of the primary response of the rat sensomotor cortex when intervals between stimuli measured 40-125 msec, whereas benactyzine reduced the late facilitation of the test response when intervals between stimuli measured 150-300 msec. No interaction could be found between benactyzine and bicuculline by this test. It was concluded from these results that the effects of bicuculline are produced by blockade of postsynaptic GABA receptors and are not connected with the activity of cholinergic structures. KEY WORDS: bicuculline; cholinergic mechanisms; GABA; convulsions; recovery cycle.

Bicuculline, which blocks postsynaptic GABA receptors [4, 5], has been widely used in recent years in neurophysiological research to identify GABA-ergic inhibitory pathways. However, on the basis of electrophysiological data, some workers have questioned the specificity of the effects of this substance [6, 8]. It has also been shown that bicuculline in vitro competitively inhibits acetylcholinesterase activity [9]. Although in the whole organism acetylcholinesterase activity is not significantly affected by bicuculline [7], some investigators consider that the effect of the drug is due, not to blockade of GABA-ergic receptors, but to potentiation of acetylcholine effects [10]. Since bicuculline can be used as a pharmacological indicator for detection of GABA-ergic inhibitory pathways, the solution to the problem of the true mechanism of action of bicuculline is of fundamental importance. A suitable method of studying this problem is to investigate the interaction between bicuculline and cholinergic and GABA-ergic drugs on the basis of behavioral and electrophysiological tests.

EXPERIMENTAL METHOD

The action of the chosen drugs on bicuculline-induced convulsions was studied in experiments on albino mice weighing 18-20 g. Benactyzine and atropine were used as M-cholinolytics and aprophen and pediphen as N-cholinolytics. All cholinolytics were injected intraperitoneally 15 min before administration of bicuculline, in the following doses: benactyzine 1 mg/kg, aprophen 5 mg/kg, and pediphen 2 mg/kg, i.e., in doses in which these drugs block the corresponding receptors in the brain [3]. To analyze the role of GABA-ergic mechanisms in the action of bicuculline, experiments were carried out to study the effects of aminohydroxyacetic acid (AHAA) and depakine (in doses of 15 and 25 mg/kg and 200 and 300 mg/kg respectively), which are inhibitors of α-ketoglutarate-GABA-transaminase (GABA-T), on bicuculline-induced convulsions. Bicuculline was injected subcutaneously in a dose of 3 mg/kg. Depakine was injected 30 min before bicuculline, AHAA 3 h before bicuculline. The intensity of the convulsions was assessed on a four-point scale (+ weak clonus, ++ clonus,

*2-diethylaminoethyl-2,2-diphenylpropionate hydrochloride.
†1,1-diphenyl-5-diethylaminopentane.

Laboratory of Pharmacology of the Nervous System, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 8, pp. 192-194, August, 1978. Original article submitted December 18, 1977.

TABLE 1. Effect of Cholinolytics on Bicuculline-Induced Convulsions

Drugs	% of animals with convuls.	Latent period of convuls., min (M ± σ)	Degree of conv. effect	Mortality, %
Bicuculline	90	7,1±3,1	23/40	30
Benactyz. + bicucul.	90	5±1,9	29/40	40
Atropine + bicucul.	90	5,7±1,1	30/40	30
Aprophen + bicucul.	100	4±0,9	30/40	50
Pediphen + bicucul.	100	5±1,1	24/40	30

TABLE 2. Protective Effect of AHAA and Depakine against Bicuculline-Induced Convulsions

Drugs	% of animals with convulsions	Degree of convul. effect, in points	Mortality, %
Bicuculline, 3 mg/kg	90	23/40	30
AHAA + bicuculline:			
15 mg/kg	70	12/40	15
25 mg/kg	40	8/40	10
Depakine + bicuculline:			
200 mg/kg	65	14/40	15
300 mg/kg	30	7/40	10

+++ tonus, ++++ tonus and death). The percentage of animals showing convulsions, the latent period of the convulsions, and the mortality also were determined. Ten mice were used in each experimental group.

In the second part of the investigation the effect of bicuculline and of the M-cholinolytic benactyzine on the recovery cycles of the primary response of the rat sensomotor cortex to sciatic nerve stimulation was studied. Benactyzine was used to test the cholinergic structures because of the greater representation of receptors of muscarinic type in the cerebral cortex [2]. Acute experiments were carried out on 26 male albino rats weighing 200-300 g. The skull was trephined under ether anesthesia on the side of recording, the sciatic nerve was dissected on the contralateral side, and a catheter was introduced into the jugular vein and a cannula into the trachea. The animal was then artificially ventilated, immobilized with suxamethonium (3-5 mg/kg, intravenously), and the anesthetic was stopped. Bipolar stimulating electrodes were applied to the sciatic nerve and covered with mineral oil. Pairs of square pulses, 1-2 V in amplitude and 300 μsec in duration, were applied to them from the output of a two-channel Physiovar stimulator (Alvar, France). The intervals between conditioning and testing pulses varied from 40 to 300 msec. The Ag–AgCl recording ball electrode was applied to the focus of maximal activity of the primary response (the reference electrode was inserted into the neck muscles). After amplification, the biopotentials were led to the input of a Nokia LP 4840 multichannel analyzer for averaging of the responses in the course of the experiments. The results of presentation of 50 pairs of stimuli were analyzed, the time quantum Δt being 1 msec and the epoch of analysis 200-400 msec (depending on the number of memory locations used). Averaged primary responses were printed out by an automatic writer. The amplitudes of the conditioning and testing responses (from the peak of the positive wave to the peak of the negative wave) were then measured and the ratio between the amplitude of the test response and the amplitude of the conditioning response for each time interval was plotted (ordinate) against the intervals between stimuli (abscissa).

EXPERIMENTAL RESULTS AND DISCUSSION

Experiments on mice showed that none of the cholinolytics tested had any protective action against bicuculline convulsions; on the contrary, a tendency was observed for the effects of bicuculline to be potentiated and the latent period of the convulsions to be shortened (Table 1).

Meanwhile after injection of the GABA deactivation inhibitors depakine and AHAA, causing the accumulation of GABA in the brain, convulsions developed in fewer animals; the intensity of the convulsions and the mortality among the animals also were reduced (Table 2).

Cholinolytics were thus unable to prevent the convulsant action of bicuculline, whereas substances causing GABA to accumulate in the brain were effective with respect to this test.

Electrophysiological experiments also demonstrated the inability of benactyzine to modify the effects of bicuculline. Under normal conditions and during the recovery cycle of the primary response of the rat sensomotor cortex two phases could be distinguished: a phase of depression of the test response when intervals between stimuli measured 100-125 msec, and a phase of its facilitation when intervals between stimuli measured 150-300 msec (Fig. 1, curve 1).

Bicuculline (0.05 mg/kg, intravenously) weakened the depression of the test response but had virtually no effect on its facilitation phase. As the writers showed previously, the GABA deactivation inhibitor depakine has the opposite effect on cortical inhibition [1]. It potentiates depression of the test response in the recovery

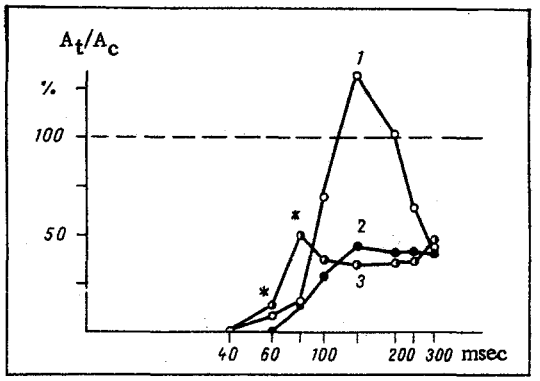

Fig. 1. Recovery cycles of primary response of rat sensomotor cortex. Results of one typical experiment are shown. Abscissa, intervals between stimuli (msec); ordinate, ratio between amplitudes of testing and conditioning responses (in %). Significance of difference between curves 2 and 3 ($P < 0.05$) marked by asterisk. 1) Control recovery cycle of primary response; 2) 20 min after injection of benactyzine (1 mg/kg, intravenously); 3) injection of bicuculline (0.1 mg/kg, intravenously) 30 min after injection of benactyzine.

cycle of the interzonal evoked potential when the testing stimulus is applied up to 100 msec after the conditioning stimulus.

Benactyzine (1 mm/kg, intravenously), unlike the GABA-ergic drugs, did not change the phase of depression of the test response when intervals between stimuli measured 40–125 msec. Its activity was manifested in another phase of the recovery cycle of the primary response: It depressed the late facilitation when the testing stimulus was applied 150–300 msec after the conditioning stimulus (Fig. 1, curve 2). No antagonism was found in the present investigation between benactyzine and bicuculline. In fact, bicuculline, if given after benactyzine, reduced depression of the test response for intervals of 40–100 msec between stimuli, but could not overcome the effect of benactyzine on the phase of latent facilitation of the test response when the interval between stimuli was 150–300 msec (Fig. 1, curve 3). Reciprocal antagonism (i.e., abolition of the effects of bicuculline by benactyzine) likewise was not observed.

The results thus suggest that the action of bicuculline in vivo is mediated not by cholinergic structures but principally through blockade of specific GABA receptors.

LITERATURE CITED

1. G. M. Molodavkin, R. U. Ostrovskaya, and V. V. Markovich, Byull. Éksp. Biol. Med., No. 2, 58 (1975).
2. R. U. Ostrovskaya, "Pharmacological study of cholinergic structures of the mesencephalic reticular formation," Candidate's Dissertation, Novosibirsk (1963).
3. A. T. Selivanova, Action of Cholinergic Drugs on Higher Nervous Activity [in Russian], Leningrad (1969).
4. D. R. Curtis, A. W. Duggan, D. Felix, et al., Nature, 226, 1222 (1970).
5. D. R. Curtis, A. W. Duggan, D. Felix, et al., Brain Res., 33, 57 (1971).
6. J. M. Godfraind, K. Krnjevic, and R. Pumain, Nature, 228, 675 (1970).
7. A. Nistri, A. M. De Bellis, E. Cammelli, et al., J. Neurochem., 23, 453 (1974).
8. D. B. Pixner, Brit. J. Pharmacol., 52, 35 (1974).
9. G. Svenneby and E. Roberts, J. Neurochem., 21, 1025 (1973).
10. G. Svenneby and E. Roberts, J. Neurochem., 23, 275 (1974).