CORTICAL INHIBITION PROCESSES AND THE ANTICONVULSANT ACTION OF BENZODIAZEPINE DERIVATIVES

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A comparative study of the effect of various benzodiazepine derivatives (clonazepam, lorazepam, diazepam, and medazepam) on the recovery cycle of the interzonal response in the motor cortex of unanesthetized immobilized cats showed that these substances selectively depress the testing potential within the interval of 20-100 msec between conditioning and testing stimuli, evidence of the potentiation of GABA-ergic inhibition in the cortex. Clear correlation was shown between the degree of protective action of the various benzodiazepine derivatives against convulsions due to GABA deficiency and their ability to induce depression of the test response. It is suggested that the GABA-positive action of the benzodiazepines plays a significant role in the mechanism of their anticonvulsant activity.

KEY WORDS: *interzonal cortical response; benzodiazepine derivatives.*

Disturbance of the normal relations between inhibition and excitation in the cortex is evidently one cause of the development of certain pathological states manifested as convulsions. An essential role in the regulation of these processes is known to be played by γ-aminobutyric acid (GABA) [3, 7]. It was shown previously that an adequate test for the investigation of GABA-ergic processes in the cortex is the recovery cycle of the interzonal response in the motor cortex [1]. Diazepam has been shown to block the phase of facilitation of the test response selectively. Analysis of this phenomenon by means of drugs with a specific effect on GABA metabolism has led to the hypothesis that it is evidently associated with potentiation of the inhibitory effect of endogenous GABA on cortical neurons.

The object of this investigation was to study whether this mechanism plays a role in the anticonvulsant action of the benzodiazepines. For this purpose various benzodiazepine derivatives were studied from the standpoint of their anticonvulsant action and their ability to potentiate inhibitory GABA-ergic processes in the cortex.

EXPERIMENTAL METHOD

Electrophysiological experiments were carried out on 48 unanesthetized adult cats, immobilized with flaxedil and artificially ventilated. The interzonal response to electrical stimulation of somatosensory area I (SI) was recorded (for details of the method, see [11]). Interzonal responses to paired stimulation (intervals between stimuli from 20 to 800 msec) were recorded on the SDR-41 tape recorder during 50 successive presentations. The responses were analyzed by the Nokia LP-4840 specialized computer.

The anticonvulsant action of the drugs was tested on mice weighing 18-20 g. Convulsions were induced by thiosemicarbazide (TSC; 18 mg/kg), a glutamate decarboxylase inhibitor, which was injected 15 min before injection of the anticonvulsants, allowing for the duration of the latent period of its action. These tests were used to investigate the benzodiazepine derivatives diazepam, lorazepam, clonazepam, and medazepam, and also anticonvulsants of other classes for comparison, namely phenobarbital and diphenylhydantoin. In experiments on cats all the drugs were injected intravenously, whereas in experiments on mice the anticonvulsants were injected intraperitoneally and the TSC subcutaneously.

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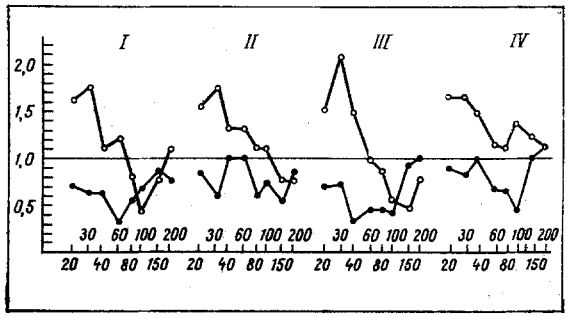

Fig. 1

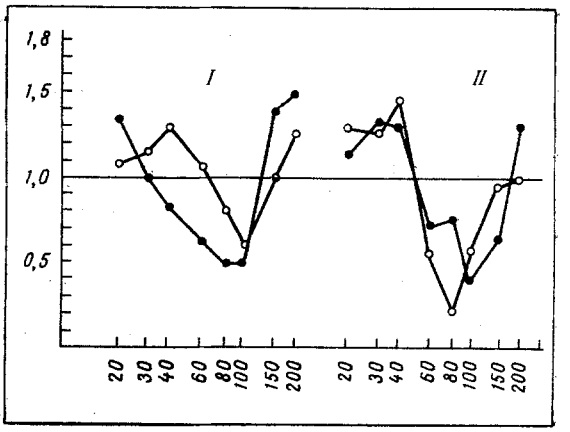

Fig. 2

Fig. 1. Comparative effects of certain benzodiazepine derivatives on recovery cycle of interzonal response. I) Clonazepam 0.1 mg/kg; II) lorazepam 0.1 mg/kg; III) diazepam 1.0 mg/kg; IV) medazepam 20 mg/kg. Abscissa, intervals between conditioning and testing stimuli (in msec; logarithmic scale); ordinate, ratio between amplitudes of testing and conditioning responses (equality of amplitudes of the two responses taken as 1). Empty circles represent recovery cycle before injection, filled circles after injection of drugs. Each point on curve is result of averaging 50 presentations of stimuli; each curve reflects results of one typical experiment.

Fig. 2. Effect of phenobarbital and diphenylhydantoin on recovery cycle of interzonal response. I) Phenobarbital 20 mg/kg; II) diphenylhydantoin 40 mg/kg. Remainder of legend as in Fig. 1.

In the electrophysiological experiments the effect of each of the substances tested was evaluated in a series of experiments on 8 animals on the basis of the threshold dose. The threshold dose was taken to be the smallest dose in which the particular substance reduced by approximately half the amplitudes of the testing response without inhibiting the conditioning response (within intervals of 20-60 msec, when maximal facilitation of the interzonal response was observed). To assess the anticonvulsant action the value of ED_{50} was calculated. The convulsants were evaluated by a four-point system.

EXPERIMENTAL RESULTS AND DISCUSSION

As was shown previously [1], the recovery cycle of the interzonal response is characterized by the following periods: by phases of early inhibition (up to 20 msec), facilitation (from 20 to 100 msec), and a weaker phase of late inhibition (from 100 to 200 msec), followed by a wave-like alternation of phases of inhibition and facilitation until complete recovery of the test response within the interval from 600 to 800 msec. Diazepam caused weakening of the phase of facilitation of the test response in doses of 0.5-2.0 mg/kg in intervals of between 20 and 80 msec between stimuli. In most experiments the threshold dose of diazepam was 1 mg/kg. Selective inhibition of the test potential within the same interval also was observed after injection of clonazepam (0.05-0.1 mg/kg) and lorazepam (0.1 mg/kg) (Fig. 1). Medazepam had a similar action on the recovery cycle of the interzonal response in a dose of 20 mg/kg. By their ability to depress the test response selectively, i.e., to potentiate cortical inhibition, these drugs could therefore be arranged in the following order of increasing activity: medazepam, diazepam, lorazepam, and clonazepam.

The compounds could be arranged in the same order with respect to their anticonvulsant action induced by deficiency of GABA formation under the influence of TSC (Table 1). It is interesting to note that although the absolute values of doses active in the two tests differed (probably as the result of the use of animals of different species and different ways of injection), the relative magnitude of the effects of the various benzodiazepine derivatives was identical or similar in the two cases.

The experiments showed that phenobarbital also can prevent convulsions induced by GABA deficiency, but in doses from 10 to 450 times greater than those of the various benzodiazepine derivatives. Diphenylhydantoin had no such effect (Table 1), although it had a marked

TABLE 1. ED_{50} of Certain Benzodiazepine Derivatives, Phenobarbital, and Diphenylhydantoin against Convulsions Induced by TSC

Drug	ED_{50}, mg/kg
Clonazepam	0,009 (0,005—0,014)
Lorazepam	0,034 (0,026—0,044)
Diazepam	0,45 (0,37— 0,54)
Medazepam	1,8 (1,24— 2,6)
Phenobarbital	15,5 (11,9—20,15)
Diphenylhydantoin	No effect under 100 mg/kg

protective action against convulsions induced by electric shock (ED_{50} = 38 mg/kg). Phenobarbital was active against both types of convulsions (ED_{50} for electric shock convulsions 66 mg/kg, for TSC convulsions 15.5 mg/kg). Phenobarbital thus possesses some degree of selectivity against convulsions caused by GABA deficiency. This conclusion was confirmed by electrophysiological experiments. Phenobarbital, in a dose of 15-20 mg/kg, was shown to reduce the amplitude of the test response (Fig. 2, I), although with ill-defined selectivity: the amplitude of the conditioning response also was reduced. After injection of smaller doses (5-10 mg/kg) there was no effect, whereas after larger doses (30 mg/kg) depression of both responses was observed. Diphenylhydantoin (20-100 mg/kg) did not weaken the facilitation of the interzonal response within the interval from 20 to 60 msec (Fig. 2, II). The role of GABA-ergic mechanisms in the realization of the effects of phenobarbital cannot thus be ruled out, but it is difficult at present to say just how important is the role of this component in its action. Data so far available on this subject are contradictory. Cutler et al. [4], for instance, found no reduction in GABA liberation in the cortex, stimulated by strophanthin C, under the influence of phenobarbital. Meanwhile, according to Costa et al. [2], phenobarbital lowers the level of cyclic guanosine monophosphate in the cerebellum, evidence of its GABA-mimetic action. To judge from the results of the present experiments, phenobarbital has a GABA-positive action but it is much weaker and less specific than that of the benzodiazepines.

The mechanism described above probably does not play an essential role in the anticonvulsant action of diphenylhydantoin although some workers do not rule out such a possibility. Raabe and Ayala [9], for instance, showed in cats anesthetized with nembutal that under the influence of diphenylhydantoin postsynaptic inhibition is potentiated in neurons of the motor cortex; however, these workers themselves consider that this effect is not necessarily a manifestation of the specific action of the drug, but could be due to its combined action with that of pentobarbital. Evidence that diphenylhydantoin has no GABA-ergic action is given by the work of Puro and Woodward [8] and also of Haefely et al. [6], who found that this substance has no effect on activity of the Purkinje cells in the cerebellum. In the light of the observed ability of penicillin to block GABA-ergic receptors [5], the ineffectiveness of diphenylhydantoin in penicillin-induced convulsions, which contrasts with the marked protective action of diazepam [10], is interesting.

It follows from the data given in this paper that there is clear correlation between the intensity of the protective effect of the various benzodiazepine derivatives against convulsions induced by GABA-negative factors and their ability to modify the recovery cycle of the interzonal response in a direction indicating a shift of excitatory and inhibitory processes in the motor cortex toward predominance of the latter. On the basis of these facts, and considering the leading role of GABA in the mechanism of inhibition in the motor cortex [3], it can be concluded that GABA-ergic mechanisms play an important role in the realization of the anticonvulsant action of benzodiazepine derivatives.

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