MECHANISM OF THE ANTICONVULSANT ACTION OF DIAZEPAM

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Diazepam is highly effective in the prevention of convulsions induced by thiosemicarbazide (TSC), i.e., due to γ-aminobutyric acid (GABA) deficiency. Electrophysiological experiments to record the recovery cycle of the interzonal response of the cat motor cortex showed that diazepam reduces the amplitude of the test response, indicating the strengthening of inhibition. This test revealed antagonism of diazepam to bicuculline, specifically blocking GABA-ergic receptors, and to TSC. Diazepam was shown to be capable of increasing the GABA concentration in the brain by inhibiting the activity of GABA transaminase in the mitochondrial fraction of brain tissue.

Key words: paroxysmal states; thiosemicarbazide; γ-aminobutyric acid (GABA); anticonvulsants (diazepam); brain mitochondria.

Tranquilizers of the benzdiazepin series, including diazepam, have recently become widely used as anticonvulsants. However, the mechanism of their anticonvulsant action has so far received little investigation. Considering data showing the important role of disturbances of γ-aminobutyric acid (GABA) metabo-

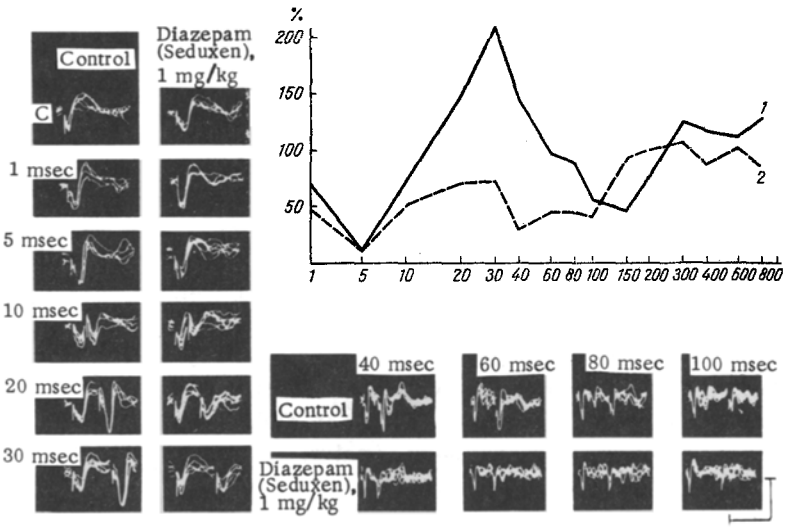

Fig. 1. Effect of diazepam (1 mg/kg) on recovery cycle of interzonal response of cat motor cortex. Graph: abscissa, intervals between conditioning and testing stimuli (in msec, logarithmic scale); ordinate, ratio between amplitudes of testing and conditioning responses (in %). Oscillographic records for intertrial intervals up to 100 msec also shown. Calibration: amplitude 600 μV, time 30 msec for intervals up to 30 msec, and 100 msec for the rest.

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lism in the genesis of epilepsy [7], it was decided to investigate the effect of diazepam on GABA-ergic processes.

An investigation was therefore carried out to study the character of the anticonvulsant effect of diazepam against seizures evoked by thiosemicarbazide (TSC), a substance that lowers the brain GABA concentration by inhibiting glutamate decarboxylase activity. Since the writers had shown previously that substances involved in GABA metabolism have a marked effect on the recovery cycle of the interzonal response of the cat motor cortex [4], it seemed advantageous to use the same method in order to study the effect of diazepam. A further object of the investigation was to study the effect of diazepam on the GABA concentration in the brain and on the activity of the chief enzyme determining the rate of its conversion, namely α-ketoglutarate-GABA transaminase (GABA-T).

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 18-22 g. TSC was injected subcutaneously in a dose of 12 mg/kg and diazepam (Seduxen) intraperitoneally in doses of 0.1-2 mg/kg 15 min after the TSC. The latent period and frequency of the seizures and the deaths were recorded. ED_{50} for diazepam was calculated by the method of Litchfield and Wilcoxon. Electrophysiological experiments were carried out on unanesthetized, curarized cats weighing 2.5-3.5 kg. The recovery cycles were investigated by paired stimulation. The arrangement of the electrodes to obtain interzonal responses of the motor cortex to stimulation of area SI and the procedure used to record their recovery cycle were described previously [3-5]. In most experiments the data were averaged with the "Neiron-1" statistical analyzer; to continue the analysis the ratio between the amplitudes of the averaged testing and conditioning responses was calculated for each intertrial interval.

The biochemical experiments were carried out on mice and rats into which diazepam was injected in a dose of 5 mg/kg 1 h before decapitation. The GABA concentration in the brain tissue was determined by paper chromatography [1]. GABA-T activity was measured on the Opton (Austria) spectrofluorometer on the basis of fluorescence of the condensation product of succinic acid semialdehyde with 3,5-diaminobenzoic acid [9], which was determined in the unpurified mitochondrial fraction (UMF) and in subcellular fractions isolated by centrifugation in a sucrose density gradient [6].

EXPERIMENTAL RESULTS AND DISCUSSION

Investigation of the effect of diazepam on seizures induced by TSC gave the following results. In a dose of 12 mg/kg, TSC evoked clonico-tonic seizures in 95-98% of the animals. From 50 to 60% of the animals died in the tonic extension phase. Starting with a dose of 0.1 mg/kg, diazepam significantly reduced the mortality, and starting with a dose of 0.5 mg/kg, it completely prevented the seizures in some animals. The effect rose in a straight line with an increase in dose (ED_{50} by this test was 0.88 mg/kg).

In small doses diazepam thus exhibited a marked protective action against seizures evoked by TSC. Comparison with its effectiveness against seizures evoked by electric shock (ED_{50} = 3.5 mg/kg) and by strychnine (ED_{50} = 44 mg/kg) [2] suggests that the effect of diazepam relative to TSC is to some extent selective. Experiments on cats showed that diazepam (1-3 mg/kg) prevents the development of paroxysmal EEG activity evoked by TSC (10 mg/kg, intravenously) and abolishes established paroxysmal activity.

Electrophysiological analysis of the effect of diazepam by recording recovery cycles of the interzonal response of the motor cortex suggested that it significantly reduces the excitability of the brain neuronal system. In the control group the recovery cycle of the interzonal response (Fig. 1) had a phase of early depression of the testing response, followed by a phase of its increase or facilitation in intervals of 20-100 msec. This facilitation was maximal in intervals of 30-50 msec, and its degree varied in different animals from 10 to 100%. Next followed phases of late depression of the test response and of late facilitation, which were weaker than the early phases. Recovery of the test response took place after 600-800 msec and the cycle in each animal was characterized by stability. Diazepam had a marked depriming effect on the facilitation phase in the interval of 20-100 msec (Fig. 1). The degree of the effect varied depending on individual sensitivity of the animal and the dose of the drug. In small doses (0.1-1 mg/kg), for instance, diazepam usually only depressed the test response to some extent, although in the most sensitive animals in the same doses it totally suppressed it. In large doses (2-10 mg/kg) it caused complete suppression of the test response and sometimes reduced the amplitude of the conditioning response. The effect of the drug began to appear after 3-5 min and it reached its maximum after 10-15 min. The duration of the effect of threshold doses of 0.1-0.3 mg/kg was 1-1.5 h, and for a dose of 5-10 mg/kg it exceeded 6 h. The action of diazepam on the

TABLE 1. Effect of Diazepam on GABA-T Activity of Subcellular Brain Fractions (M ± m)*

Conditions	UMF	Subcellular fractions				
		A (myelin fragments)	B	C	D	E ("pure" mitochondria)
			synaptosomal fractions			
Control	308± 64	133± 26	86± 13	78± 4	59± 10	475± 60
Diazepam	217± 59	110± 27	75± 13	68± 8	56± 2	292± 73
% inhibition	29,4	17,3	13	13,2	5,7	39,5

*In μg/g of succinic acid semialdehyde formed.

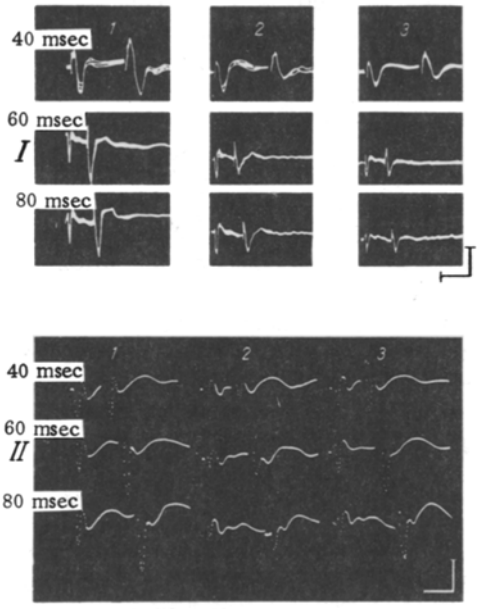

Fig. 2. Interaction between effects of diazepam 5 mg/kg and caffeine 40 mg/kg (I) and between diazepam 1 mg/kg and bicuculline 0.15 mg/kg (II): 1) control; 2) diazepam; 3) caffeine (or bicuculline) with diazepam. Numbers on left of records show intertrial intervals. Time calibration in Fig. 2, I is 20 msec for interval of 40 msec, and 80 msec for the rest; in Fig. 2, II it is 60 msec. Amplitude calibration 500 μV in I and II.

recovery cycle was abolished by bicuculline, a specific blocking agent of GABA-ergic structures. In small doses (0.1-0.15 mg/kg) it restored the amplitude of the test response in the interval 20-100 msec to its initial value, and in cases in which the conditioning response was depressed bicuculline restored its amplitude also (Fig. 2). Diazepam (0.5-1 mg/kg) abolished the facilitation of the test response evoked by TSC (5-7 mg/kg). Since bicuculline and TSC behave as antagonists to diazepam in preparoxysmal doses, intensifying EEG activity, the action of another activating substance (caffeine), not interfering with GABA-ergic processes, was studied on the recovery cycle when modified by diazepam. The results showed that caffeine (20-40 mg/kg) does not abolish the depression of the test response, whereas the conditioning response remained unchanged or was increased (Fig. 2). As regards the selective depression of the test response and its antagonism to bicuculline and TSC the effect of diazepam was analogous to the effect, described by the writers previously [4] of depakin,* an antiepileptic drug, which increases the endogenous GABA con-

*Sodium n-dipropylacetate (translator's note).

centration by inhibiting GABA-T activity.

Investigation of the endogenous GABA content in the brain showed that its level was significantly increased from 208±7 to 280±39 μg/g wet weight of tissue by diazepam in a dose of 5 mg/kg, the increase being significant. Whereas TSC reduced the brain GABA concentration from 208± 7 to 140± 13 μg/g, a combination of diazepam with TSC produced a smaller decrease, to 180± 13 μg/g only.

It will be clear from Table 1 that diazepam inhibited GABA-T activity and that this effect was most marked in the subcellular fraction of "pure" mitochondria. In agreement with data in the literature [8], in these experiments it was this subfraction that showed the highest initial GABA-T activity (57.2%); the remaining 42.8% of the total activity was distributed among subfractions A, B, C, and D.

Presumably inhibition of GABA-T activity and the consequent accumulation of GABA are among the causes of the reduction in the excitability of the brain structures produced by diazepam and reflected in the increased inhibition revealed in electrophysiological experiments and in the protective effect against seizures evoked by TSC and bicuculline. The presence of inhibitory effects of small doses of diazepam, not changing GABA concentration in the brain tissue, may perhaps be explained on the grounds that in these doses the drug modifies intracellular transport and binding of GABA. This hypothesis that GABA-ergic processes participate in the mechanism of the effects of diazepam is in harmony with data showing that the drug can potentiate presynaptic inhibition in the spinal cord [11], for these processes also are known to be mediated by GABA.

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