QUANTITATIVE HISTOCHEMICAL STUDY OF THE EFFECT OF BENZODIAZEPINE TRANQUILIZERS ON GABA-TRANSAMINASE ACTIVITY IN THE RAT BRAIN

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The effect of diazepam and nitrazepam in doses of 0.5, 1, 2.5, and 5 mg/kg on GABA-transaminase activity in the cerebellar cortex and hippocampus of rats was studied by a quantitative histochemical method. Inhibition of GABA-transaminase was found under the influence of both substances. The decrease in activity was particularly marked in the hippocampus after administration of diazepam. It is suggested that the changes observed are the result of inhibition of the enzyme and also of a decrease in the rate of GABA turnover.

KEY WORDS: benzodiazepine tranquilizers; GABA-transaminase; quantitative histochemistry.

Evidence has recently been obtained that the anticonvulsant, muscle-relaxant and also, possibly, the psychotropic effect of the benzodiazepine tranquilizers is linked with their effect on the γ-aminobutyric acid (GABA) system [3-7, 9, 14]. One way by which the GABA-positive effect may be achieved is through inhibition of GABA-transaminase (GABA-T), an enzyme splitting GABA [3, 4]. It is considered that only high doses of diazepam (5 mg/kg or more) have this effect. However, the effects of lower doses of the drug cannot always be demonstrated by biochemical investigation of large areas of the brain. Histochemical methods are often more informative. Despite this fact, the method of histochemical detection of GABA-T developed by Van Gelder [13] is virtually never used in pharmacology. The first attempts to use this reaction to study the action of neurotropic drugs were based on visual assessment of activity of the enzyme [8].

The objects of the present investigation was a quantitative histochemical study of the effect of benzodiazepine tranquilizers on GABA-T activity, the method being based on the cytospectrophotometric investigation of the kinetics of this reaction undertaken previously [2].

EXPERIMENTAL METHOD

Experiments were carried out on 40 male albino rats weighing 150-200 g. The cerebellar cortex and hippocampus, structures with relatively high GABA-T activity [12], were investigated. Diazepam and nitrazepam were injected intraperitoneally in doses of 0.5, 1, 2.5, and 5 mg/kg, made up in starch mucilage; injection of 0.5 ml starch mucilage alone served as the control. The animals were decapitated 40 min after the injection. Portions of the cerebellum and hippocampus were isolated from the brain and frozen in iso-octane, cooled with liquid nitrogen. GABA-T was detected by Van Gelder's [13] and Ritter's [10] method in the writer's own modification [2] on frozen sections, 10 μ thick, through the cerebellum and hippocampus. The sections were incubated for 40 min at 43°C in medium of the following composition: GABA 2.9×10 M, α-ketoglutarate-Na_2 $5.2 \cdot 10^{-3}$ M, NAD $3 \cdot 10^{-3}$ M, tetranitroblue tetrazolium $2.4 \cdot 10^{-4}$ M, phenazine metasulfate $3 \cdot 10^{-5}$ M, NaCN $1 \cdot 10^{-3}$ M, malonic acid $9.6 \cdot 10^{-3}$ M, dextran 60 mg/ml; the medium was made up in Van Gelder's buffer [13] and the final volume was 20 ml. To allow for nonspecific reduction of tetrazolium, parallel tests were carried out in the presence of $1.1 \cdot 10^{-3}$ M aminoacetic acid, a GABA-T inhibitor. The sections were mounted in glycerol-gelatin gel and subjected to photometry by the plug method at 546 nm, with a probe 1 μ in diameter. Activity of GABA-T was estimated from the increase in optical density compared with sections incubated in the presence of inhibitor, and expressed in optical density units. With each dose of the compound, at least 300 measurements were made. The significance of differences and correlation between dose and effect were determined by statistical methods [1].

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TABLE 1. Effect of Benzodiazepines on GABA-T Activity (M ± m)

Drug	Dose, mg/kg	GABA-T activity, optical density units	
		cerebellum	hippocampus
Control	—	0,242±0,005	0,096±0,004
Diazepam	0,5	0,240±0,005	0,058±0,003*
"	1	0,224±0,004*	0,053±0,003*
"	2,5	0,178±0,004*	0,047±0,003*
"	5	0,150±0,004*	0,008±0,003*
Control	—	0,230±0,006	0,092±0,003
Nitrazepam	0,5	0,205±0,004*	0,079±0,003*
"	1	0,193±0,004*	0,075±0,003*
"	2,5	0,185±0,003*	0,065±0,003*
"	5	0,148±0,003*	0,047±0,002*

* $P<0,001$.

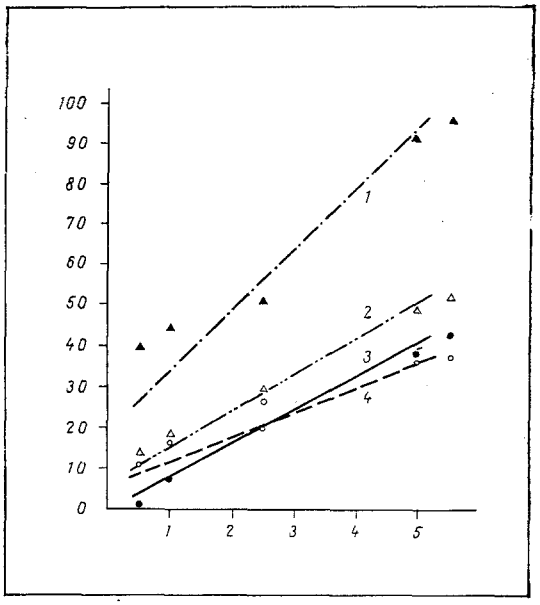

Fig. 1. Decrease in GABA-T activity under influence of benzodiazepines. Abscissa, dose of drug (in mg/kg); ordinate, degree of inhibition of GABA-T activity (in %); straight lines represent theoretical regression lines; 1 and 2) effect of diazepam and nitrazepam respectively on hippocampal neurons; 3 and 4) the same, on cerebellar cortical neurons.

EXPERIMENTAL RESULTS

Visual assessment of the intensity of the histochemical reaction showed high GABA-T activity in the Purkinje cells of the cerebellar cortex and moderate activity in the pyramidal neurons of the hippocampus, in agreement with data in the literature [11]. These structures were selected for quantitative histochemical analysis, and the pyramidal neurons of the dorsal and ventral hippocampus were exposed separately to photometry. No significant differences were found between the GABA-T activity of the dorsal and ventral areas of the hippocampus.

Administration of diazepam and nitrazepam caused inhibition of GABA-T activity in both structures (Table 1).

As Fig. 1 shows, the degree of depression of enzyme activity caused by diazepam in a dose of 5 mg/kg in the cerebellar cortex corresponded to the values obtained by Ostrovskaya et al. [5]. The dynamics of changes in GABA-T activity under the influence of nitrazepam in the cerebellar cortex and hippocampus and

TABLE 2. Correlation between Dose of Drug and GABA-T Activity

Drug	Cerebellum			Hippocampus		
	r	*t*	*P*	*r*	*t*	*P*
Diazepam	—0,78	3,62	<0,001	—0,57	2,05	<0,05
Nitrazepam	—0,82	4,01	<0,01	—0,63	2,46	<0,05

Note. r) Coefficient of correlation; t) coefficient of significance.

under the influence of diazepam in the cerebellar cortex was similar. Changes induced by diazepam in the hippocampus were distinguished by much greater intensity.

The relationship between GABA-T activity in the structures studied and the dose of the drugs was inversely proportional in character. The numerical values of the coefficients of correlation indicate that correlation between them was sufficiently close (Table 2).

The most likely cause of the decrease in GABA-T activity induced by benzodiazepine tranquilizers is inhibition of the enzyme. Evidence in support of this conclusion is given by the accumulation of GABA in the brain after administration of high doses of diazepam [7,12]. However, the differences in the dynamics of inhibition of GABA-T in the cerebellum and hippocampus under the influence of diazepam suggest that inhibition of GABA-T is not the only factor reducing its activity. Diazepam, in a dose of 1 mg/kg or higher, reduces the rate of GABA turnover in the brain [9]. A decrease in the rate of secretion of the mediator which is the substrate of GABA-T may be an additional cause of the reduction in its activity.

The effect of the benzodiazepines on GABA-T is thus manifested even in small doses, evidence of the selectivity of their influence on structures possessing high activity of GABA-ergic processes.

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