Consequently, unlike antidepressant activity, in the manifestation of which an important role is played by the histidyl-proline complex, a more important role in realization of the learning-stimulating action of TRH and related dipeptides is played by preservation of the structure of pyroglutamate. The derivatives of pyroglutamic acid studied, irrespective of whether a third amino acid was present or absent, or whether GABA or β-alanine was used as the second amino acid, had a facilitating effect on the learning ability of rats in the CPAR. It must be emphasized that the stimulating effect of pyroglutamate derivatives on learning is not combined with stimulation of locomotor activity, as it is in the action of the classical psychostimulants. It can accordingly be concluded that the pyroglutamate derivatives studied in this investigation have a selective effect on learning ability.

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EFFECT OF BENZODIAZEPINES ON AMP-DEAMINASE AND ADENOSINE-DEAMINASE ACTIVITY IN RAT BRAIN TISSUE *IN VIVO*

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KEY WORDS: benzodiazepines; diazepam; phenazepam; rat brain; AMP deaminase; adenosine deaminase.

With the widespread use of tranquilizers of the 1,4-benzodiazepine series (diazepam, phenazepam,* etc.) in clinical practice the need has arisen for elucidation of the molecular mechanisms of their action.

Since benzodiazepines have no direct action on synaptic transmission, according to data in the literature their effects are linked with an indirect effect on inhibition in the CNS through GABA-ergic mechanisms [4, 6, 13]. It has been suggested that endogenous benzodiazepines of purine nature may be formed in nerve cells, and in particular, metabolic products of adenosine-5'-monophosphate (AMP) and adenosine itself, namely inosinic acid, inosine, and

*7-Bromo-1,3-dihydro-5-(2'-chlorophenyl)-2H-1,4-benzodiazepin-2-one.

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TABLE 1. AMP Deaminase and Adenosine Deaminase Activity in Rat Brain Tissue (in μ-moles NH_3/mg protein) after Administration of Benzodiazepines (M ± m)

Enzyme	Control	Benzodiazepine injected	Time after injection, h			
			1	2	3	6
AMP deaminase	0,341±0,021	Diazepam	0,644±0,027*	— *	0,455±0,027*	0,400±0,030
		Phenazepam	0,405±0,012*	0,462±0,032	0,319±0,017	—
		Diazepam	0,433±0,027*	— *	0,381±0,029	0,349±0,049
Adenosine deaminase	0,328±0,017	Phenazepam	0,323±0,036	0,454±0,021	0,424±0,035*	0,319±0,015

*P < 0.5 compared with corresponding control.

Legend. Conditions of incubation of AMP deaminase: K-succinate buffer 0.05 M (pH 6.5), KCl 0.16 M, AMP 0.005 M, 15% brain homogenate 0.4 ml, total volume of sample, 1 ml; incubation for 40 min at 37°C. Conditions of induction of adenosine deaminase: K-phosphate buffer 0.02 M (pH 7.2), KCl 0.16 M, adenosine 0.005 M, 15% brain homogenate 0.4 ml, total volume of sample 1.4 ml; incubation for 120 min at 37°C.

hypoxanthine [11], which can evidently function as endogenous ligands of benzodiazepine receptors.

Recent investigations have shown that purinergic mechanisms play an important role in the realization of effects of benzodiazepines [9]. Competitive relationships have been found between benzodiazepines and adenosine for receptor binding sites in synaptosomes [8] and benzodiazepines have been shown to inhibit adenosine uptake [9].

The writers showed previously [1] that benzodiazepines significantly inhibit activity of brain 5'-nucleotidase — a fragment located mainly on cell membranes.

The aim of the present investigation was to study the effect of benzodiazepines on activity of AMP deaminase and adenosine deaminase, cytosol enzymes which catalyze reactions of hydrolytic deamination of AMP and adenosine, with the formation of inosinic acid and inosine, respectively.

EXPERIMENTAL METHOD

Experiments were carried out on 200 noninbred male albino rats weighing 200 g, which received an intraperitoneal injection of either phenazepam or diazepam, tranquilizers of the benzodiazepine series. The Soviet preparation phenazepam was injected in a dose of 5 mg/200 g body weight. For this purpose the dry phenazepam powder was suspended in physiological saline with the addition of the surfactant Span-80 (Loba Chemie, Austria). The stable suspensions contained 2.5 mg of the dry substance in 1 ml. Tests were carried out 1, 2, 3, and 6 h after injection of phenazepam.

Diazepam was used in the form of ampules of Seduxen solution containing 5 mg of the active substance in 1 ml (from Gedeon Richter, Hungary). Diazepam was given in a dose of 3 mg/200 g body weight. Activity of the enzymes was determined 1, 3, and 6 h after injection of the drugs.

Control animals received an intraperitoneal injection of 0.6-2.0 ml of 0.9% NaCl solution. From eight to 12 animals were used in each series.

AMP deaminase [7] and adenosine deaminase [3] activity was determined in brain tissue homogenates. Activity of both enzymes was judged from the quantity of ammonia formed, which was estimated by a modified Conway's microdiffusion method [2].

EXPERIMENTAL RESULTS

Data on the effect of benzodiazepines on AMP deaminase activity in the rat brain are given in Table 1 and Fig. 1A. Activity of this enzyme showed a sharp increase (by 89.1%) 1 h after injection of diazepam, and AMP deaminase activity still remained raised (by 33.4%) after 3 h. Enzyme activity was back to normal 6 h after injection of the benzodiazepine.

Changes in AMP deaminase activity followed a similar time course after injection of phenazepam. For instance, 1 h after injection of the drug enzyme activity was increased a little (by 18.7%), and after 2 h it was increased by 35.5%. AMP deaminase activity returned to its original level 3 h after injection of the drug.

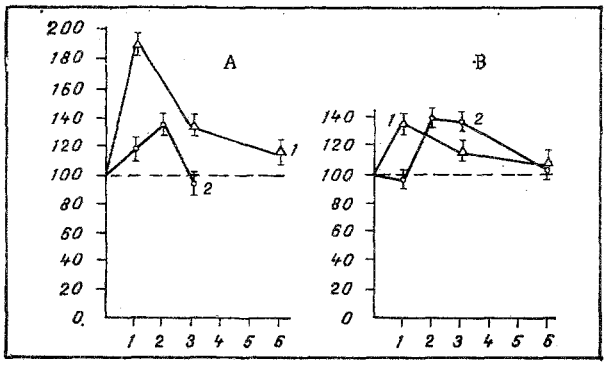

Fig. 1

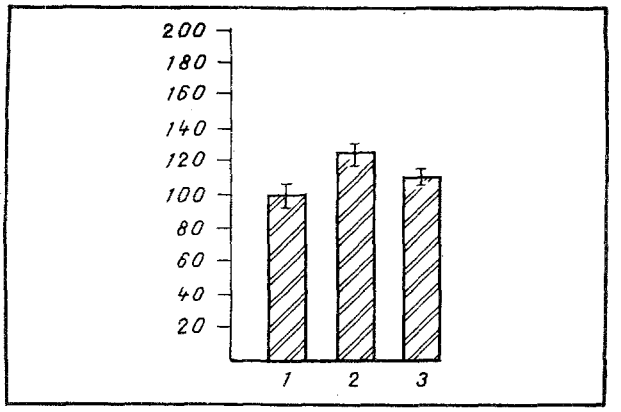

Fig. 2

Fig. 1. Changes in AMP deaminase (A) and adenosine deaminase (B) activity after injection of benzodiazepines. Abscissa, time after injection (in h); ordinate, change in activity (in %). 1) Diazepam, 2) phenazepam. Values obtained in animals receiving 0.9% NaCl taken as 100%.

Fig. 2. Effect of actinomycin D on induction of AMP deaminase activity by diazepam (1 h after injection). 1) 0.9% NaCl, 2) diazepam, 3) diazepam + actinomycin D (100 μg). Ordinate, change in activity (in %).

Phenazepam caused an increase (by 38.5%) in enzyme activity 2 h after injection. This activating effect of phenazepam still persisted (by 29.2%) 3 h after injection. After 6 h the adenosine deaminase activity did not differ significantly from the control.

To determine whether the increase in activity of these enzymes was due to the direct action of the benzodiazepines or to a chain of neurochemical changes following injection of these drugs, a series of experiments was conducted *in vitro*, in which rat brain homogenates were incubated for 1 h at 37°C with shaking, in the presence of 500 μg diazepam (diazepam was added to the control samples after the reaction had ended). Determination of AMP deaminase activity in these tests revealed a marked increase (by 80.2%): 0.340 ± 0.072 μmole NH_3/mg protein in the control, 0.618 ± 0.074 in the experiment; $P < 0.02$).

To discover what causes the increase in deamination of adenylic acid and adenosine, i.e., whether it is due to activation of the enzymes mentioned or to an increase in their synthesis de noto, a series of experiments was conducted in which benzodiazepines were injected into animals and enzymic ammonia formation was subsequently determined after injection of actinomycin D, an inhibitor of DNA-dependent RNA synthesis. In these experiments, 1 h before injection of diazepam, a solution of actinomycin D (from Reanal, Hungary), was injected intraperitoneally in doses of 100 and 150 μg/200 g body weight. Actinomycin D and 0.9% NaCl solution were injected into control animals at the corresponding times.

A weak tendency for AMP deaminase activity to increase (by about 20%) was still observed 1 h after injection of diazepam preceded by actinomycin D in a dose of 100 μg/200 g body weight, but in a dose of 150 μg/200 g actinomycin D abolished the increase in AMP deaminase activity induced by diazepam almost completely (by 89.1%).

These results are evidence that benzodiazepines intensify the de novo synthesis of enzymes responsible for purine deamination, as a result of which the intracellular concentrations of inosine and inosinic acid rise.

Recent investigations have demonstrated the sedative, anxiolytic, and muscle-relaxing actions of adenosine [5, 10]. Since the brain, unlike peripheral tissues, possesses high adenosine deaminase activity, Skolnick et al. [12], attempted to use inosine as an anticonvulsant. These workers reported that intraventricular injection of inosine lengthened the latent period of appearance of metrazol-induced convulsions by 2-4 times.

The rapid increase in de novo synthesis of AMP deaminase and adenosine deaminase found in rat brain tissue in response to administration of benzodiazepines suggests that the neurochemical mechanism of the action of benzodiazepines may be mediated through their influence on purine metabolism. In particular, the sedative (anticonvulsant) effect of benzodiazepines

may be associated with the more rapid breakdown of adenine derivatives of the purines to inosinic acid, to inosine, and also possibly, to hypoxanthine.

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INHIBITION OF NORADRENALIN DEAMINATION BY VARIOUS ANTIDEPRESSANTS

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Changes in metabolism of serotonin or noradrenalin (NA) in the brain play an important role in the mechanism of action of antidepressants [8]. Such changes may be the result of disturbance of biosynthesis, storage, or (and) deamination of the amines. Incomplete correlation between inhibition of monoamine oxidase (MAO) and amine accumulation in the brain [6] may be due to the multiplicity of forms (heterogeneity) of MAO, the key enzyme of amine catabolism. According to the binary classification, MAO exists in the form of two types: A and B. Serotonin and NA are specific substrates of type A MAO, which is sensitive to low concentrations of chlorgyline [10]. Much greater inhibition of deamination of NA than of serotonin has been demonstrated under the influence of pyrazidol (pirlindol), an inhibitor of type A MAO, on rat brain mitochondria [4]. The possible existence of an independent binding site (or form of enzyme), responsible for NA deamination in the human brain [11], was indicated previously.

Imipramine, a reuptake inhibitor, inhibited deamination of serotonin and 2-phenylethylamine in the brain [2, 9], but the effect of this drug, and also of other antidepressants that are inhibitors of monoamine reuptake on NA deamination has not been investigated.

For the reasons described above, it was decided to study the effect of antidepressants with different structure and type of action on enzymic deamination of NA in the brain.

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