Vol. 94, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 16, 1980 Pages 849-854

EFFECT OF IMIPRAMINE HYDROCHLORIDE ON THE ACTIVITY OF GAMMA-AMINOBUTYRIC ACID TRANSAMINASE IN REGIONS OF THE RAT BRAIN

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Received April 25,1980

Summary

The effect of intraperitoneal injection of imipramine hydrochloride on the activity of gamma-aminobutyric acid transaminase was determined in three regions of the rat brain.

The cerebral hemispheres did not show a significant change in the activity of gamma-aminobutyric acid transaminase. Cerebellum and brain stem, both, however, showed a very significant decrease in the activity of the enzyme at 15 and 30 minutes after drug administration. At 90 minutes after drug administration, the activity of gamma-aminobutyric acid transaminase had returned to nearly control values.

Introduction

Gamma-aminobutyric acid transaminase, GABA-T (4-aminobutyrate : 2-oxoglutarate aminotransferase, EC 2.6.1.19), is one of the three enzymes of the GABA shunt. In the CNS of mammals as well as the peripheral nerves of arthropods, GABA shunt represents an alternate pathway to the portion of the tricarboxylic acid cycle that leads from alpha-oxoglutarate to succinate (1). GABA is intimately related to the oxidative metabolism of carbohydrates in the CNS by means of this shunt. GABA is also an inhibitory neurotransmitter in both vertebrate and invertebrate nervous systems.

The major degradative pathway for GABA is transamination by GABA-T, and it can be metabolized by both central and peripheral tissues. The properties and distribution of brain GABA transaminase have been studied in detail (2,3). The kinetic data of the enzyme has also been reported (4).

0006-291X/80/110849-06$01.00/0

Since imipramine hydrochloride is a very well known antidepressant, its effect on the enzyme GABA-T which degrades GABA and regulates the steady-state concentration of GABA, was thought to be an important aspect for study. A differential effect of the drug on the enzyme with time was observed in different areas of the brain.

Materials and Methods

Experimental Animals Adult rats of Holtzman strain weighing between 200-250 g were used for experimental purposes.

Chemicals Chemicals used were from Sigma Chemical Co., U.S.A., unless otherwise specified.

Imipramine hydrochloride treatment Imipramine hydrochloride (Suhrid-Geigy Ltd., India) was injected intraperitoneally (50 mg/Kg body weight) to the rats. Since the imipramine hydrochloride was anaqueous solution, control rats were also injected with the same amount of distilled water.

Preparation of Homogenate Rats were killed by cervical dislocation and brains were excised and chilled immediately. Cerebral hemispheres, cerebellum and brain stem were separated and weighed immediately. Homogenates were prepared (1:10) using a Potter Elvehjem type homogeniser fitted with a teflon plunger. The homogenising medium contained the following in the final concentrations; 0.25 M sucrose, 20 mM triethanolamine buffer pH 7.4 and 0.1 mM dithiothreitol.

Estimation of GABA-T activity GABA-T was estimated essentially according to the method of De Boer and Bruinvels(5). A unit of GABA-T activity was defined as one μmol of NADH formed at 37°C in one hour per g fresh weight of tissue.

Statistical Analysis To express significance, 2-way analysis of variance was applied. For difference between control and treated groups, Dunnette's multiple comparison test was applied.

Results

The effect of imipramine hydrochloride on the activity of GABA-T was determined in three regions of the rat brain, at six different time intervals. The results, calculated as a change in percentage activity, taking controls as 100% are shown in Fig. 1. The cerebral hemispheres did not show a significant change in the activity of GABA-T at all the six time intervals studied.

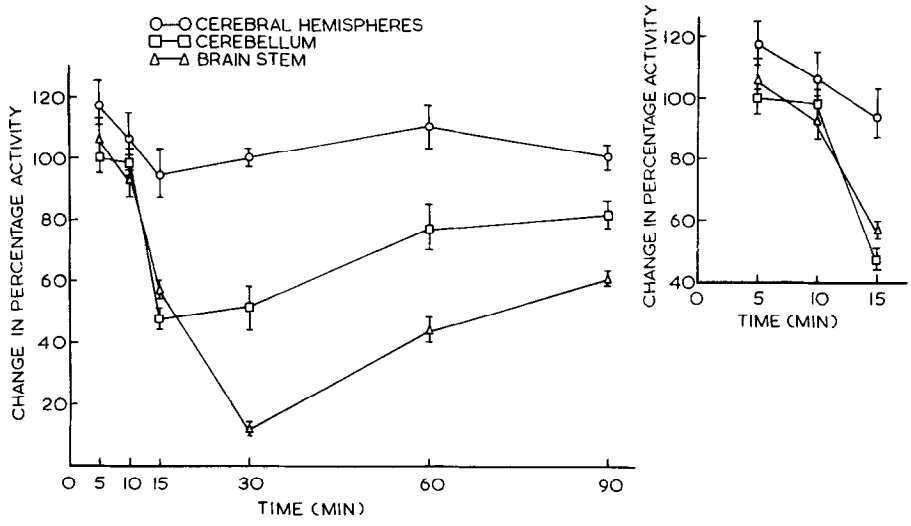

FIG. 1. Effect of imipramine (5 mg/100 g) on GABA-T activity after different time of administration (taking control as 100%).

Cerebellum and brain stem, both, however, showed a very significant decrease in the activity of the enzyme at 15 and 30 minutes after drug administration respectively. The maximum effect of the drug in the cerebellum was at 15 minutes and in the brain stem at 30 minutes (Fig. 1). By 90 minutes the activity in the cerebellum had returned to 82% of the control activity and that of brain stem to 61% of the control activity.

The changes in the activity of GABA-T at different time intervals in different regions of the brain, with significance is given in Table 1.

Discussion

Imipramine hydrochloride (Tofranil, Depsonil) is a widely used tricyclic antidepressant drug. It is chemically similar to the phenothiazines, and it has been found useful as an anti-depressant (6,7,8). Imipramine and other anti-depressants may be effective clinically to some extent, but their biochemical mechanism of action is not well elucidated.

TABLE - 1 EFFECT OF IMIPRAMINE ON GABA-T ACTIVITY AFTER DIFFERENT TIME OF ADMINISTRATION*

TIME IN MINUTES	CEREBRAL HEMISPHERES µmoles/g/h	p value	CEREBELLUM µmoles/g/h	p value	BRAIN STEM µmoles/g/h	p value
Zero	20.3 ± 0.8		23.5 ± 2.0		27.7 ± 1.5	
5	22.4 ± 1.6	N.S.	24.5 ± 0.5	N.S.	26.7 ± 2.7	N.S.
10	20.3 ± 0.5	N.S.	24.0 ± 2.4	N.S.	23.5 ± 1.1	N.S.
15	18.2 ± 1.4	N.S.	11.7 ± 0.6	***	14.5 ± 0.1	***
30	20.4 ± 0.4	N.S.	12.1 ± 1.8	**	3.2 ± 0.0	***
60	22.4 ± 1.3	N.S.	18.3 ± 2.3	N.S.	12.3 ± 1.0	***
90	20.3 ± 0.8	N.S.	19.2 ± 1.1	N.S.	17.1 ± 0.4	***

* - Concentration of imipramine used per rat was 5 mg/100 g body weight. Each value is a mean of 4 to 6 values.

N.S. - Not Significant; versus control.

** - $p < 0.01$; versus control.

*** - $p < 0.001$; versus control.

The effectiveness of imipramine has been shown to be related to the route of administration. The appearance of desmethyl imipramine in brain, the only pharmacologically active metabolite of imipramine, occurred only after intraperitoneal or oral administration of the drug (9,10,11).

The uptake of GABA has been reported to be inhibited by imipramine (12). Monoamine oxidase inhibitors, clinically used as antidepressants reduce the activity of GABA-T and markedly elevate cerebral GABA concentrations (13).

The rapid return of the activity of GABA-T, in the present series of experiments between 30 and 60-90 minutes (Fig. 1) may be due to the unavailability of GABA in vivo in the specific cell compartment where GABA-T is localized. The enzyme,therefore, may undergo a reversible conformational change (due to substrate unavailability) at 15 and 30 minute time intervals when the maximum concentration of imipramine is reached in the brain (14). Enzyme activity then returns to control levels after the effect is decreased at 90 minutes, due to rapid metabolism of the drug in the liver after intraperitoneal administration (15).

Although in the completed assay system excess GABA (as substrate) has been added, the modified enzyme, after imipramine treatment does not elicit the total activity, suggesting the uneven distribution of either imipramine or one of its metabolites at 15 and 30 minute intervals in different regions of the brain. This may explain the variation in enzyme activity observed in isolated areas of the brain i.e. the cerebral hemispheres, cerebellum and brain stem (Table 1). The heterogeneous pattern of the effect of the drug in the brain regions could also be due to the differential localization of GABA (16,17,18), highest amounts being found in cerebellum and brain stem.

Further work on the changes in the concentration of GABA with the drug and the effect on other enzymes of the GABA shunt is in progress in our laboratory to find out the actual molecular mechanism of action of the drug.

Acknowledgements We thank Suhrid-Geigy Ltd., India, for the kind gift of imipramine hydrochloride. M.R.P. acknowledges the financial aid from the Council of Scientific and Industrial Research in the form of a Senior Research Fellowship. The technical assistance of Shri Basant Lal Sharma is greatly appreciated.

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