Technology Section

Pak. j. sci. ind. res., vol. 38, no. 7, July 1995

ENHANCEMENT OF BRAIN REGIONAL TRYPTOPHAN CONCENTRATION FOLLOWING ADMINISTRATION OF DIAZEPAM: A DOSE RELATED STUDY

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(Received July 17, 1994; revised February 27, 1995)

Administration of diazepam at doses of 1,3 and 5 mg/kg attenuated home cage activity and open field activity of rats. Plasma concentrations of total tryptophan decreased and those of free tryptophan increased in diazepam injected rats. The concentration of tryptophan increased in the hypothalamus, striatum and cortex but not in the brain stem of diazepam injected rats. The increases in the hippocampus were only marginal and occurred at high doses of the drug. The possible mechanism for the increased availability of tryptophan to the brain and decreased serotonin turnover reported in diazepam injected rats is discussed.

Key words: Diazepam, Tryptophan, Serotonin.

Introduction

A role of central serotonin, (5-hydroxytryptamine, 5-HT), a biogenic amine in the development of anxiety is often suggested in both neurochemical and behavioral studies, [1,2]. Several investigators have shown that administration of benzodiazepines, the well-known anxiolytics, decreased the turnover of 5-HT in the central nervous system [3-11]. This view has been confirmed by the finding that administration of diazepam diminished the formation of endogenous 5-hydroxtryptophan (5-HTP) in the brain [12].

L-Tryptophan, an essential amino acid is the precursor of serotonin in the brain, for which the initial source is dietary. The tryptophan available for the synthesis of 5-HT comes from the periphery. Increased brain concentration of L-tryptophan have been reported following the administration of benzodiazepines [8,13]. *In vitro* investigations have suggested that this is due to increased uptake of L-tryptophan [14]. Pratt *et al.* [8] found that administration of clonazepam increased 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and tryptophan concentrations in mice.

The uptake of tryptophan from blood to brain is brought about via a carrier-mediated transport mechanism [15] shared by all large neutral amino acids (LNAAs). Plasma concentrations of total [16] free [17] tryptophan and LNAAs [18] have been shown to affect brain tryptophan concentration in different physiological [19] and pharmacological conditions [20].

Despite several studies showing decreased metabolism of 5-HT following diazepam administration [3-11], effects of diazepam on plasma and brain tryptophan concentrations have not been reported. The present study is therefore designed to investigate the effects of various doses of diazepam on plasma total, free and brain regional tryptophan concentrations. Effects of diazepam on home cage and open-field activity are also determined.

Materials and Methods

Animals. Locally bred male Wistar rats weighing 150-200g, on arrival, purchased from PCSIR Laboratories Pakistan were housed individually in a quiet room with free access to cubes of standard rodent diet and water for at least 4-5 days before experimentation.

Chemicals. Diazepam (F. Hoffmann La-Roche and Co. Ltd., Basel Switzerland) available in ampules of 5 mg/ml was used in the experiment. Other chemicals were purchased from Sigma Chemical Company (USA) or BDH Chemical Pool (England).

Drugs and injections. Animals were randomly assigned to control and test groups. Diazepam available in 5 mg/ml ampules, diluted to required concentrations in 0.9% NaCl w/v, were injected intraperitoneally in volumes of 1, 3 and 5 mg/kg/ml body weights. Control animals received an equal volume of NaCl (0.9% w/v). Four groups of rats were used each time in a balanced design.

Behavioural studies. Home cage activity. Transparent perspex 26x26x26cm cages were used to individually cage the animals. Rats injected with various doses of diazepam or saline were observed in their home cages 15 min. post injections for 5 min. to monitor activity. During this time period number of cage crossings, climbings and groomings were counted.

Open-field activity. The open-field apparatus used in the present investigation consisted of a square area 76x76cm with walls 42 cm high. The floor was divided by lines into 25 equal squares. Rats injected with 1, 3 and 5 mg/kg/ml diazepam or

vehicle were exposed to the open-field 30 min. later. To determine open-field activity a saline or drug injected rat was placed for the first time in the centre square of the open-field apparatus. The number of squares crossed in the open-field, with all four paws were scored for 5 min. as described earlier [21].

Decapitation and brain dissection. Animals were decapitated 1 hr. after the injectioins of saline or various doses of diazepam to collect blood in heparinized centrifuge tubes. Blood was centrifuged to obtain plasma. The brain taken out immediately, placed on an ice-cold glass plate was dissected in regions with the help of brain slicer. Hypothalamus, striatum, cortex, hippocampus and brain stem were dissected out from the brain as described earlier [22] and were rapidly stored at -70°C until analysis. Liver was also removed and stored for the estimation of tryptophan.

Biochemical estimations. Plasma total and free (ultrafilterate) tryptophan, liver tryptophan and brain regional tryptophan were all determined by the flourimetric method by Denckla and Dewey [23] as modified by Bloxam and Warren [24].

Statistical analysis. Behavioural data were statistically tested by Mann Whitney U-test. Biochemical data were analysed by one-way ANOVA. Posthoc comparison was performed by Newman-Keuls statistics.

Results and Discussion

Figure 1 shows that i.p. administration of diazepam at doses 1, 3 and 5 mg/kg decreased open-field locomotor activity of rats. Maximum decreases were produced at 3 mg/kg. The decreases at a higher dose (5 mg/kg) were smaller and less significant than the decreases observed at 3 mg/kg.

Figure 2 shows that administration of diazepam at doses of 1,3 and 5 mg/kg also decreased home cage activity of rats. The decreases by 3 doses used were comparable.

Table 1 shows that acute administration of various doses of diazepam decreased total tryptophan concentration in plasma and increased the concentrations of free tryptophan. The concentration of tryptophan in liver was also increased by these doses of the drug. While these doses produced non-significant effect on the concentration of glucose.

Table 2 shows that administration of 1 mg/kg diazepam increased tryptophan concentration in the hypothalamus and striatum only. At doses of 3 mg/kg the increases were significant in the hypothalamus, striatum and cortex. At doses of 5 mg/kg, drug administration increased tryptophan concentration in the hypothalamus, striatum, cortex and hippocampus. The levels of tryptophan in the brain stem were not altered by these doses of drug.

Our findings that acute administration of diazepam at doses of 1,3 and 5 kg/mg decreases open-field locomotion is consistent with previous studies [25]. In addition, we find

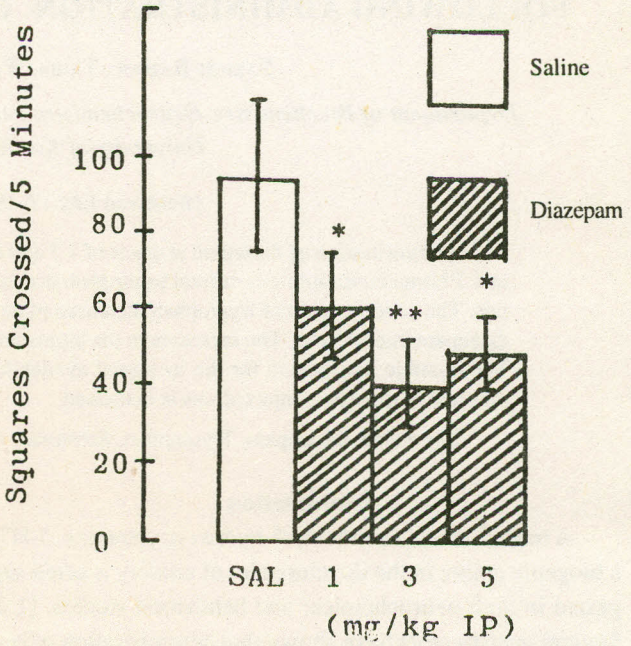

Fig.1. Open-field activity of saline and diazepam injected rats during 5 min. exposure to the open-field. Values are means ± SEM (n=6) 30 min. after the drug or saline administration. Significant differences by U-test, *P=0.05, **P=0.025.

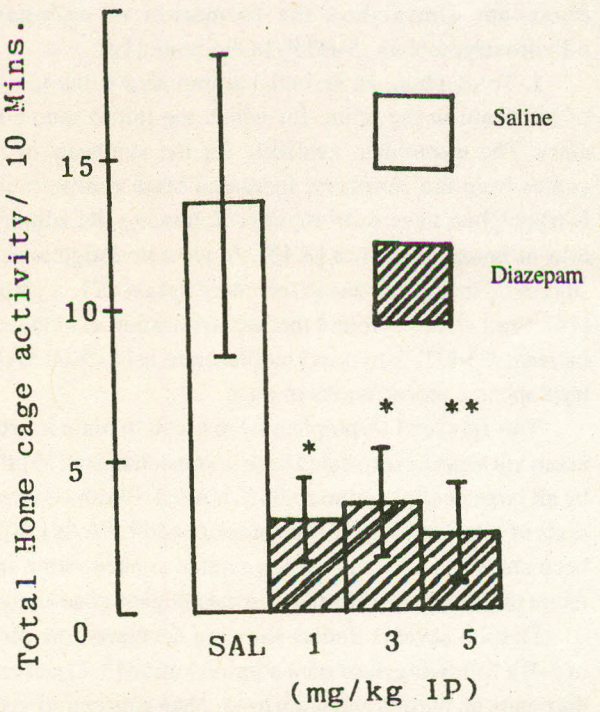

Fig.2. Home cage activity of saline and diazepam injected rats during 10 min. observation period. Values are means ±SEM (n=6) 15 min. after the drug or saline administration. Significant differences by U-test. *P<0.05, **P<0.025.

that home cage activity is also decreased. The observed decreases of both open-field and home cage activity may be due to sedative effects of the drug. The present study therefore shows that benzodiazepines (BZs) unlike caffeine [26] may produce sedation both in home as well as novel environment.

A number of neurochemical investigations have shown that systemic administration of BZs decreases 5-HT turnover [3-11] and this correlates with the antianxiety effects of the drug [4,27]. Brain tryptophan hydroxylase, the rate-limiting enzyme of 5-HT biosynthesis, exists unsaturated with its substrate tryptophan [28-30]. Therefore, it would be expected that decreased 5-HT turnover following the administration of BZs may be associated with decreased brain concentrations of tryptophan. However, the present study shows that brain concentrations of tryptophan were decreased in many brain regions. This suggests that administration of diazepam may inhibit the activity of tryptophan hydroxylase. This view is consistent with the finding that diazepam diminished the formation of endogenous 5-hydroxytryptophan (5-HTP) in the brain [12].

TABLE 1. THE EFFECT OF DIAZEPAM ON PLASMA TOTAL, FREE, LIVER TRYPTOPHAN CONCENTRATION (ug/ml), AND GLUCOSE CONCENTRATION. (mg/dl) IN CONTROLS AND DIAZEPAM TREATED RATS 1 HOUR AFTER INJECTIONS.

	Saline	Diazepam 1mg/kg	Diazepam 3mg/kg	Diazepam 5mg/kg	1-ANOVA df=3,20	
		**	**	**		
Total Trp.	14.6±0.9	8.6±1.4	6.2±1.2	5.2±1.1	F=82.0	P<0.01
		**	**	**		
Free Trp.	1.8±0.2	3.2±0.8	4.1±0.9	4.7±1.0	F=15.0	P<0.01
Glucose	33±3.4	32±5.0	32±4.0	32±4.1	F=0.3	n.s.
		**	**	**		
Liver.	11±17.98	23.60±66.36	22.80±23.60	28.85±73.78	F=12.7	P<0.01

**P<0.01 from respective saline injected rats by Newman-Keuls statistics following one-way ANOVA, Values are mean ±S.D. (n=6)

Regional variations have been observed in the effects of diazepam on brain 5-HT synthesis. Thus after systemic administration of moderate doses (5 mg/kg) of diazepam, 5-HT turnover was preferentially decreased in the hipppocampus, but not in the striatum and cortex [31]. In the present study the increases of tryptophan concentrations more marked in the striatum and hypothalamus were only marginally increased in the hippocampus only at doses of 5 mg/kg. The cortical levels of tryptophan were also increased but significantly only at doses of 3 and 5 mg/kg, while the concentrations of tryptophan in brain stem did not increase.

The regional differences in the effects of diazepam on brain tryptophan concentrations may be explained by (1) an uneven cerebral distribution of the drug; (2) a difference in the density of benzodiazepine receptors; (3) regional variations in the interaction of diazepam with other neuronal systems which are involved in the modulation of serotonergic neurotransmission.

A further consideration is that how an increase in brain tryptophan concentration occurs following the administration of diazepam? Tryptophan from the circulation is transported to the brain by a carrier mediated transport mechanism [15]. Plasma concentrations of total [16], free [17] tryptophan and large neutral amino acids (LNAAs) [18] have been shown to modulate brain tryptophan concentrations in different pharmacological [20] and physiological [19] conditions.

Coassolo *et al.* [32] studied the binding of L-tryptophan and chlorazepate (a benzodiazepine) to human serum albumin (HSA) by microcalorimetery and concluded that the two ligands are specific competitors for the same primary binding site.

The observed increases of brain tryptophan concentration in the present study may be due to increased plasma concentrations of free tryptophan because plasma total tryptophan decreased. Plasma concentrations of LNAAs were not determined in the present study. However, it is less likely that

TABLE 2. THE EFFECT OF VARIOUS DOSES OF DIAZEPAM ON BRAIN REGIONAL TRYPTOPHAN CONCENTRATION. (ug/ml) IN CONTROLS AND DIAZEPAM TREATED RATS 1 HOUR AFTER THE INJECTIONS.

	Saline	Diazepam 1mg/kg	Diazepam 3mg/kg	Diazepam 5mg/kg		1-ANOVA df=3,20
	**	**	**			
Hypothalamus	1.4 ± 1.83	2.6 ± 5.60	3.93 ± 3.87	5.66 ± 8.18	F = 66.2	P < 0.01
Hippocampus	2.3 ± 3.87	3.2 ± 7.02	2.67 ± 4.21	3.915 ± 8.15	F = 7.2	P < 0.01
Striatum	3.9 ± 6.34	6.4 ± 18.7	9.80 ± 2.26	15.588 ± 2.210	F = 43.4	P < 0.01
Cortex	3.7 ± 4.11	5.0 ± 10.0	6.479 ± 1.880	9.710 ± 1.144	F = 26.5	P < 0.01
Brain Stem.	6.6 ± 11.85	7.9 ± 19.7	5.545 ± 1.916	6.341 ± 1.607	F = 2.1	n.s.

*P<0.05, **P<0.01 from respective saline injected rats by Newman-Keuls Q-statistics following one-way ANOVA. Values are means ± S.D. (n=6). Diazepam was administered as described in methods.

change in LNAAs concentration occurs because plasma concentrations of glucose were not altered [33].

The important finding of the present study is that brain tryptophan concentrations are not decreased by diazepam administration. The reported decrease of cerebral 5-HT turnover following diazepam administration [3-11] may occur because of the changes in the kinetics of regulatory enzyme of 5-HT biosynthesis.

Acknowledgement. The authors wish to thank INFAQ BCCI Foundation for giving scholarship to Miss Farhat Batool for carrying M. Phil/Ph.D in the Department of Bio-Chemistry, University of Karachi.

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