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INCREASED PRECURSOR AVAILABILITY DID NOT INCREASE FOOD INTAKE AND 5-HT TURNOVER RATE IN THE HYPOTHALAMUS OF DIAZEPAM INJECTED RATS

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ABSTRACT

In view of a possible role of serotonin (5-hydroxytryplamine 5-HT) in regulation of appetite and anxiety, the effects of 1,3 and 5mg/kg doses of diazepam on brain serotonin precursor and effects of single and repeated diazepam (1 mg/kg 2* daily for 4 days) administration on hypothalamic 5-HT, 5-hydroxyindoleacetic acid (5-HIAA and 5-HIAA/5-HT ratio are investigated in rats. Daily diazepam treatment decreased food intakes. Diazepain injected rats exhibited a dose-dependent increase of tryptophan in the hypothalamus. Administration of diazepam (1 mg/kg) to 4 day saline injected rats on the 5th day in-creased 5-HT and decreased 5-HIAA levels in the hypothalamus. 5-HIAA/5-HT level also decreased. 4 day diazepam injected rats injected with saline on the 5th day also e^{x}hibited similar changes of 5-HT. 5-HIAA and 5-HIAA/5-HT ratio. Administration of diazepam to 4 day diazepam injected rats again decreased 5-HIAA concentrations but did not in-crease 5-HT levels in the hypothalami of rats. Possible mechanism involved in the anorectic effects of diazepam-induced changes of hypothalamic 5-HT turnover rate is discussed.

INTRODUCTION

Central serotonin (5-hydroxytryptamine; 5-HT), one of the principal monoamine in the brain, is thought to be important in the development of anxiety and stress (Baldwin D. 1995). An extensive animal literature links brain monoaminergic system to the regulation of appetite and eating behaviour (Curzon G. 1991). The serotonergic and dopaminergic system have been reported to play a key role in the control mechanism involving appetite inhibition (Blundell J.E., 1991). A number of studies have shown that manipulations which tend to increase 5-HT functions in the synaptic cleft decrease food intake of experimental animals (Kennett G.A., 1988, Haleem D.J., 1996).

Benzodizepines (BZs), the well-reputed anxiolytics, are particularly effective in stimulating feeding of rats or mice that were offered a highly palatable, but novel food in a novel environment (Hie S.E., 1980). Diazepam is generally termed as “food intake stimulant”. The hypothalamus is believed to be the site of brain transducing satiety signals of serotonin (Haleem D.J., 1993). The region of the brain is also known to have a key role in neuroendocrine regulations (Humbert T., 1994).

The present study is, therefore, designed to monitor changes of food intakes and corresponding changes of 5-HT and 5-HIAA in the hypothalamus of diazepam injected rats.

MATERIALS AND METHOD

Animals and Treatment:

Locally bred male Albino Wistar rats weighing 180-200gm purchased from PCSIR Laboratories Pakistan were housed individually under a 12th light: dark cycle in a quiet room with free access to cubes of standard rodent food and water atleast 4 days before experimentation.

Diazepam (F. Hoffmann-La Roache Ltd, Basel Switzerland) available in 5mg/ml ampoules, diluted to required concentrations in saline (0.9% NaCl w/v) was injected i.p. Control animals received saline injection at the same time.

Effects of Diazepam on Hypothalamic Tryptophan Concentration:

Rats injected with 1,3 and 5mg/kg diazepam or saline were decapitated 1h later and hypothalami dissected out (Haleem D.J., 1994) were stored for the fluorimetric determination of tryptophan (Denckla ER., 1967, Bloxam D.L., 1974).

Effects of Single and Repeated Administration on Hypothalamic 5HT Metabolism:

Daily diazepam treatment was performed by injecting the drug at doses of 1 mg/kg 2* daily for 4 days between 9:00-10:00 h and 17:00-18:00 h. Control animals were injected with saline at the same times. Food intakes were measured daily each morning before injecting the drug. Daily food intakes are shown as g/100g body weight. After 4 day drug administration, effects of diazepam challenge (1mg/kg) on hypothalamic 5-HT metabolism were determined on the 5th day. Diazepam at doses of 1mg/kg was injected to a group of 4 day saline injected and another group of 4 day diazepam injected rats. A group of 4 day saline and another group of a 4 day diazepam injected rats received saline injection. Animals were decapitated 1h later and hypothalami dissected out (Haleem DJ., 1994) on ice were stored at 70°C for the determination of 5-HT, 5-HIAA and 5-HIAA/5-HT ratio by high performance liquid chromatography with electrochemical detection (HPLC-EC; Haleem DJ., 1996).

Statistical Analysis: Data on the effects of various doses of diazepam on hypothalamic tryptophan concentration were analysed by one way analysis of variance (ANOVA). Effects of single and repeated diazepam administration on hypotahalamic 5-HT, 5-HIAA and 5-HIAA/5-HT ratio were analysed by two way ANOVA. Repeated measure design was adopted for the data on daily changes of food intakes. Posthoc comparisons were done by Newman-keuls statistics. P values > 0.05 were considered insignificant.

RESULTS

Fig. 1 shows the effects of diazepam injected at doses of 1.3 and 5mg/kg on hypothalamic levels of tryptophan. ANOVA (df-3,20) revealed a significant treatment effect on hypothalamic ($F=66.2$, $P<0.01$) tryptophan levels. Posthoc comparisons showed that the levels of tryptophan in the hypotalami increased significantly ($P<0.01$) at all the doses of drug used.

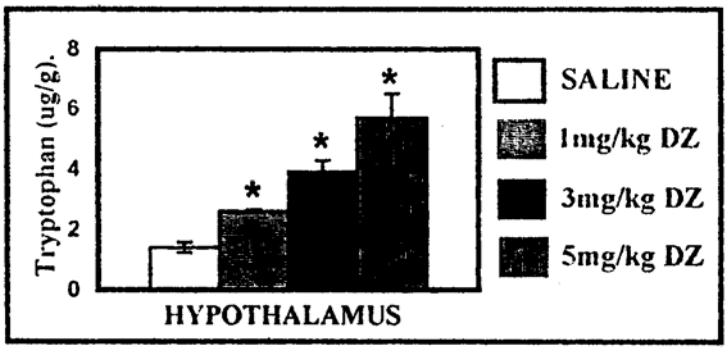

Fig. 1: Effects of diazepam (1,3 & 5mg/kg) on hypothalamic tryptophan concentration. Values are means ± S.D. (n=6). One hour alter the drug or saline administration. Significant differences by Newman-Keuls statistics' $P<0.01$ from saline injected rats following one-way ANOVA.

Fig. 2. Shows the effects of repeated diazepam administration on daily changes of food intakes. Analysis by two way ANOVA (repeated measure design) showed a significant effect of drug ($F=14$, df 1,22, $P< 0.01$), daily administration ($F=5.2$, df 1.22, $P< 0.01$) and a significant interaction ($F=40.5$, df 1,22, $P < 0.01$) between two factors for daily changes of food intakes. Posthoc comparisons showed that diazepam administration at doses of I mg/kg 2* day decreased daily food intake values during the 4 day treatment.

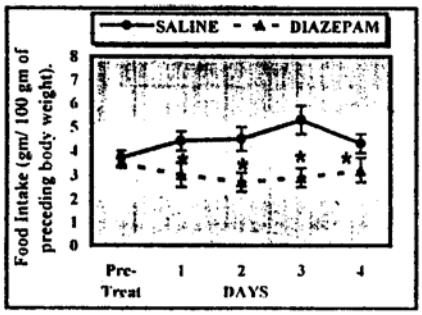

Fig 2. Effects of diazepam (1mg.kg 2* daily) on daily changes of Food Intakes. Values are means ± S.D. (n=12). Significant differences by Newman-Keuls statistics *P following two-war ANOVA (repeated measure design).

Fig. 3: Shows the effects of I mg/kg diazepam challenge on hypotahalamic 5-HT, 5-HIAA and 5-HT ratio in 4 day saline and 4 day diazepam injected rats. Two way ANOVA showed significant single (F=6,6, df 1, 20, P < 0.05) and insignificant repeated (F=3.0, df 1, 20, P> 0.05) administration effect and insignificant interaction (F-4.0, df 1, 20 P > 0.05) between two factors for 5- HT. Significant single IF- 108.7, df 1. 20. P = 0.01) and repeated (F=143.0, df 1, 20 P < 0.0) administration effect and an insignificant interaction (F 0.8, df 1, 20, P > 0.05) was observed for 5-HIAA. Data on 5-HIAA/5-HT ratio showed significant single (F=37.3, df 1, 20, P<0.01) and repeated (F=35.6, df 1,20, P < 0.01) administration effect and significant interaction (F=6.3, df 1.20 P < 0.05) between two factors.

Posthoc comparisons showed that administration of diazepam to 4 day saline injected rats increased 5-HT and decreased 5-HIAA concentrations. 5-IIIAA S-HT ratio also decreased. 4 day diazepam injected rats injected with saline on the 5th day also exhibited enhanced 5-HT levels and reduced 5-HIAA levels and a decrease in 5-I IIAA/5-HT ratio. Diazepam injected to 4 day diazepam injected rats resulted in further decrease of 5-HIAA levels and 5-HIAA/5- HT ratio but 5-HT levels did not increase.

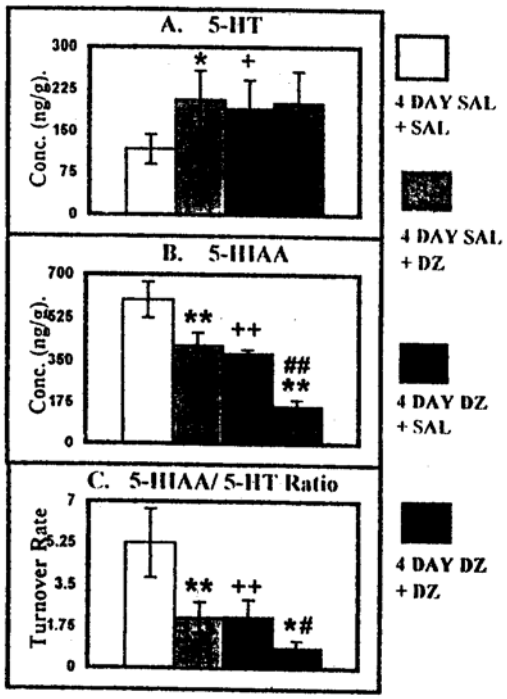

Fig. 3: Values are means + S.D. (n=6). Significant difference by Newman-Keuls statistics following two-way ANOVA. *P<0.05, **5 P<0.01 from respective saline injected rats +P<0.05,++P<0.01 from 5 day saline injected rats. #P < 0.05, ##P< 0.01, from 4 day saline injected rats injected with diazepam of the 5th day.

DISCUSSION

Although a role of the hypothalamus in the anti-anxiety effects of BZs has not been reported, however, this region of the brain is known to have a key role in the regulation of appetite and eating behaviour (Leibowtiz S.F., 1990). Pharmacological manipulations which tend to increase 5-HT functions in the hypothalamus are anorexiogenic (Haleem D.J., 1993). Decrease of daily food intakes in diazepam injected rats as observed in the present study (Fig 2) may therefore occur due to enhanced 5-HT function at local hypophagic sites (Kennett G.A., 1988).

The present study shows that administration of diazepam resulted in dose-dependent increases in the concentration of tryptophan (Fig 1) and 5-HT (Fig 3) in the rat hypothalami. These data are in general agreement with previous reports (15) that both single and repeated administration of diazepam leads into an increase in the content of the brain seratonin precursor tryptopan (VaIzelli S., 1990) and in the brain levels of serotonin (Stancheva S., 1988; Farhad Batool 1999). The present study shows that these effects of diazepam on hypothalamic 5-HT concentration could be observed even 24th after the last administration. It is possible to relate the elevation of 5-HT with both increased precursor availability and decreased release of the neurotransmitter because single administration *of* diazepam increased precursor concentration (Fig 1) for the neurotransmitter synthesis and both single and repeated administration of diazepam decreased 5-HIAA concentration (Fig 3). Decreases of 5-HIAA and 5-HT ratio but not the increases of 5-HT were more pronounced in the hypothalami of 4 day diazepam injected rats re-injected with diazepam on the 5th day.

In conclusion, the present study shows that systemically administered diazepam increases hypothamic tryptophan concentration. Diazepam-induced changes of hypothalamic 5-HT metabolism may well be involved in the anorectic and neuroendocrine effects of the drug.

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