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EFFECT OF CHLORPROMAZINE ON BRAIN BIOGENIC AMINES IN NORMAL AND HYPERGLYCEMIC STATE

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ABSTRACT

In present study the effects of chlorpromazine (CPZ) were studied on NA, DA and its metabolities i.e. DOPAC and HVA as well as on 5-HT and its metabolite 5-HIAA in normal and hyperglycemic rats. Significant differences were noted in brain biogenic amines of normal and diabetic control animals. In case of CPZ treated normal and hyperglycemic rats significant differences were observed only in case DOPAC and 5-HIAA. NA and 5-HIAA levels were significantly affected by CPZ and diabetes interaction.

INTRODUCTION

Phenothiazines as a class, and especially Chlorpromazine (CPZ) are among the most widely used antipsychotic drugs in the practice of psychiatric medicine today. Among phenothiazines, CPZ markedly possess an extra-ordinarily large number of actions i.e.,antipsychotic, tranquilizing, adrenolytic, antiemetic, and potentiation of a large number of hypnotics (Mahju 1971).

CPZ is the first phenothiazine derivative which showed the uniqe type of central depressant action i.e., tranquilization. Therefore even today it is the most widely studied antipsychotic drug for its physiological, biochemical and psychological actions. There is more information available pertaining to its actions than any other drug used in psychiatric treatment.

The first attempts to treat mental illness with CPZ alone were made in Paris in 1951. Delay and Deniker (1952) found that CPZ achieved more than symptomatic relief of agitation or anxiety and that it had an ameliorative effect upon psychotic process with diverse symptomology. Lehman and Hanrahan (1954) for the first time reported the use of CPZ in psychotic excitement and manic states.

Biomedical investigations revealed a wide variety of effects on brain metabolism. CPZ is a lipid soluble, surface active substance, accumulates in the lipoproteins of the cell membranes where it may influence cell permeability and therefore transport across the membrane. It can also alter enzyme substrate reactions and release coupled reactions from control (Bradley, 1963). CPZ also interferes with carbohydrate metabolism (Baldessarini, 1996). In present study the effects of CPZ were studied on different brain biogenic amines and their metabolites in normal and hyperglycemic rats.

MATERIALS AND METHOD

Materials

Chlorpromazine [Largactil from Rhone-Poulenc]. Alloxan tetrahydrate [Fluka Chemica]
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Animals

Locally bred albino female Wistar rats weighing about 150-250g were used. Before conducting the experiment, the animals were given a period of acclimatization of at least five days to the laboratory conditions with 12h 1ighVdark cycle. Food and water was given *ad libitum.*

Experimental protocol

There was one normal and one diabetic group and within each group there were test and control animals. The control animals of both groups received saline (0.9% NaCl), while the test group was given CPZ in a dose of 0.7mg/Kg. Control and test animals were decapitated after 2 hours (approx.) of receiving the injection. Brain samples were stored at -70°C, for analysis by HPLC-EC (High performance liquid chromatography with Electrochemical detector).

Experimental diabetes

For the induction of diabetes, Alloxan (150mg/Kg) was used by the method described elsewhere by Akhtar et al (1981). The rats which exhibited blood sugar level 200-350mg/dl were considered diabetic.

Brain Dissection

After decapitation, brain was taken out from the cranial cavity over an ice plate within 30 seconds, and dipped in chilled saline to remove the traces of blood. Then it was transferred carefully into a glass vial and stored at-70°C, until assay of neurochemicals by HPLC-EC.

Assay of Biogenic Amines

Brain samples were extracted as described earlier by Haleem et al 1992, 1990). Determinations were made by HPLC-EC at an operating potential of 0.8 V (glassy carbon electrode Vs Ag/AgC1 reference electrode). A Nova-Pak ODS, 3.9x150mm separation column was used. The solvent system was methanol (18%), octyle sodium sulphate. (0.023%), and EDTA (0.0035%) in 0.1M phosphate buffer (pH 2.9).

Statistical Analysis

Neurochemical data was analyzed by Two-Way ANOVA. Intergroup comparisons were made by using Newman-keuls statistics. p values, < 0.01, < 0.05 were considered as highly significant and significant respectively.

RESULTS AND DISCUSSION

CPZ had significantly reduced the NA (Nor-adrenaline) levels in both normal and diabetic test animals (Table-1). But the reduction in NA levels of normal test animals is much more than diabetic test animals. CPZ also affected the DA (Dopamine) levels insignificantly in both normal and diabetic test animals. This reduction of NA and insignificant effect on DA by the CPZ could be due to the inhibition of enzyme tyrosine hydroxylase, which catalyzes the rate limiting step i.e., the hydroxylation of tyrosine, in the biosynthesis of catecholamines (Zigmond et al. 1989). Previously it had been observed that inhibition of this enzyme by amino acid analogues such as a-methyl tyro-sine leads to depletion of catecholamines in brain and various sympathetic nerves (Wesley et al. 1988). The levels of NA in diabetic test animals were also significantly affected by CPZ and diabetes interaction.

The insignificant effect of CPZ on DA levels could be due to he fact that whole brain is used in the present study. Previously it had been observed that CPZ-HCI (1-60mg/kg i.p) given to rats had increased NA and DA in the dierrcephalon and amygdala. But it did not increase DA at any other brain region examined. Thus the effects of CPZ on central amine metabolism are different de-pending upon the site of the brain and monoamine metabolities (Kazushi et al. 1979).

It was noted that the DA levels were significantly higher in diabetic control animals than in normal control animals. The diabetic test animals also showed higher levels of DA as compared to normal animals. Two-way ANOVA also showed significant effect of diabetes on the levels of DA. This observation is consistent with the previous observations.

Earlier work had shown that the rate of DA turnover in the limbic forebrain in diabetic mice was significantly higher than in non- diabetic mice. There could be increased DA neurotransmission, which might be due to increase in the DA release in mesolimbic DA systems (Junzo 1994). It was observed that the DA receptors were increased in rats made diabetic by alloxan *(Lozovsky et al,* 1983). It was also known that the chronic hyperglycemia exerts selective effects in the nigrostriatal dopaminergic system in rats. These selective effects *may* result in increased number of dopaminergic binding sites in the striatum (Semi et al. 1985).

So the increase in the number of DA receptors and dopaminergic binding sites may cause increase DA neuronal activity which resulted in increased levels of DA in diabetic control animals.

In diabetes there is derangement of glucose metabolism which may affect the release of different catecholamine neurotransmitters. The work of Ian Sub et al. (1993) showed the effects of glucose on the catecholamine release from the hypothalamic fragments in vitro. It was found that glucose modulates the catecholamine release through a direct action on the catecholinergic nerve terminal as well as through a trarissynaptical action. The glucose modulation of the catecholamine release may explain at least in part the diabetes induced changes in the hypothalamic catecholamine metabolism. It is also suggested that untreated diabetic state is associated with progressive impairment of neurotransmitter release (Broderick 1989).

CPZ had increased the DOPAC (Dihydroxy-phenyl acetic acid) and HVA (Homovanilic acid)

levels significantly in normal animals and only HVA levels in diabetic animals (Fig. 3 & 4), which is consistent with the observation of Lewis (1983), who reported that the administration of CPZ resulted in increased concentration of acidic metabolities of dopamine i.e. DOPAC and HVA.

CPZ had increased the DOPAC levels significantly in normal test animals, but it had produced almost no effect on DOPAC levels of diabetic rats. Lack of effect on DOPAC levels of diabetic animals could be due to the fact that DA metabolism in regions with high and low DA contents are differentially affected in diabetes since different neuronal processes are altered in each DA brain area (Martin et al., 1995). There was insiginificant effect of CPZ on DOPAC levels of diabetic test animals, however they had lower levels as compared to normal test animals. While the levels of HVA were significantly higher in diabetic test animals as compared to control.

In present study the CPZ did not produce any significant changes in DA and DOPAC levels of diabetic animals. This lack of effect by CPZ on DOPAC levels of diabetic animals could be due to decrease deamination of DA. There was significant increase in levels of HVA and insignificant decrease in the levels of DOPAC of diabetic control when compared to normal controls indicating the increased 0-methylation of DA into HVA. This reduced deamination and increased methylation in diabetic animals could be due to altered central dopaminergic transmission. Lozovsky et al (1981) had indicated that central dopaminergic transmission might be altered in diabetes mellitus.

CPZ had insignificantly decreased the 5-HT levels in both normal and diabetic rats. Previously it had been observed that CPZ-HCI (1-60mg/kg i.e.) given to rats increased the serolonin in brain stem and cerebral cortex, but it did not increase serotonin at any other brain region examined. Thus the effects of CPZ on central amine metabolism are different depending upon the site of the brain and monoamine metabolities (Kazushi et al., 1979).

So the insignificant decrease in 5-HT levels after administration of CPZ is explainable in terms of increase occurring only in 5-HT rich regions.

The comparison of normal and diabetic control groups showed that the diabetic animals had higher levels of 5-HT. The diabetic test animals also had significantly higher levels of 5-HT as compared to normal test animals. The increase in 5-HT levels of diabetic animals is consistent with the previous observations which showed the elevated 5-HT release after electrochemical signals in rats made diabetic with streptozocin (Broderick 1989).

The CPZ had insignificantly increased the 5-HIAA in normal animals indicating increased 5-HT metabolism. This could be due to stimulatory effects on monoamine oxidaes. In diabetic animals CPZ had significantly reduced the 5-HIAA levels as compared to diabetic controls. Two-way ANOVA showed that there was significant interaction between CPZ and diabetes regarding the levels of 5-HIAA. So the significant decrease in 5-HIAA levels showed by diabetic test animals could be due to the effects of diabetes as well as due to drug and diabetes interaction. The diabetic controls had higher levels of 5-HIAA than normal controls. This increase in 5-HIAA levels indicates the increase turn over of 5- HT in diabetic animals which is consistent with the earlier observations of Broderick et al. (1989).

It could be stated that CPZ had acted in a similar manner on biogenic amines and their metabolities, except DOPAC and 5-HIAA in normal and diabetic state.

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Table-1
Effect of Chlorpromazine on Brain Biogenetic Amines in Normal & Diabetic Rats

	Normal		Diabetic		Two-Way Anova		df(1,20) CPZ & Diabetes Interaction
	Control (Saline)	Test	Control (Saline)	Test	CPZ	Diabetes	
NA	629.66 ± 117.74	217.85 ± 63.54**	681.09 ± 86.27	516.19 ± 59.32**$^{++}$	F=6913 p< 0.01	F=25.43 p< 0.01	F=12.67 p< 0.01
DA	217.63 ± 44.07	176.48 ± 129.34	516.13 ± 94.15^{++}	544.69 ± 113.82^{++}	F=1.49 p = N.S	F=97.81 p< 0.01	F=1.35 p = N.S
DOPAC	274.21 ± 82.07	355.48 ± 44.40*	252.00 ± 67.98	251.36 ± 32.1^{+}	F= 2.70 p< N.S	F= 6.36 p< 0.05	F= 2.78 p= N.S
HVA	49.42 ± 19.63	77.52 ± 07.84*	71.06 ± 24.57^{+}	97.59 ± 17.10^{+}*	F=18.77 p< 0.01	F=10.95 p< 0.01	F=0.01 p= N.S
5-HT	135.39 ± 40.44	108.59 ± 27.54	285.19 ± 62.55^{++}	275.08 ± 29.58^{++}	F=1.13 p= N.S	F=85.53 p= 0.01	F= 0.22 p= N.S
5-HIAA	303.38 ± 48.21	369.28 ± 72.39	441.07 ± 36.00^{++}	331.92 ± 51.88**	F= 0.96 p< N.S	F= 5.19 p< 0.05	F= 15.8 p< 0.01

Values are mean ± S.D. (n=6). Significant differences by Newman-Keuls test *p< 0.05, **p< 0.01 as compared to saline injected rats, $^{+}$p<0.05, $^{++}$p<0.01 as compared to normal rates following Two-Way ANOVA df (1,20).

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