Behavioral deficits in rats following acute administration of glimepiride: Relationship with brain serotonin and dopamine

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Abstract: A considerable body of literature suggests that depression and diabetes mellitus are co-morbid. The present study was designed to test any possible behavioral deficits and/or neurochemical changes in the brain as induced by the anti-diabetic drugs. Twenty-four rats were divided into four groups: (i) saline (ii) glimepiride (2.5mg/kg)- (iii) glimepiride (5.0mg/kg)- and (iv) glimepiride (10 mg/kg) injected animals. Behavioral activities in Skinner's box, open field and elevated plus maze were monitored 20, 35 and 45 minutes post injection respectively. Animals were decapitated 60 minutes post injection to collect brain samples. Samples were kept at -70ºC until neurochemical analysis by HPLC-EC. Results from the present study show decreased time spent in the open arm of the elevated plus maze ($p<0.05$) at all the three doses. A decrease in the HVA (Homovanillic acid) levels at all three doses ($p<0.01$) was also observed along with decreased 5-HT (5-Hydroxytryptamine) ($p<0.05$ at 5.0 and 10mg/kg) and 5-HIAA (5-Hydroxyindoleacetic acid) ($p<0.05$ at all three doses) levels. Since a decrease in 5-HT metabolism can induce depression-like effects, the present study therefore suggests that the occurrence of depression in diabetic patients is due to the use of glimipride. Effects of long-term administration of smaller doses of glimipride are to be explored further to monitor tolerance in glimipride-induced deficits of serotonin. The finding may help to explore the cause of depression in diabetics for improving pharmacotherapy in diabetes.

Keywords: Behavioral activities, diabetes, depression, glimepiride, neurotransmitters.

INTRODUCTION

Diabetes Mellitus is one of the commonest endocrine disorders, characterized by hyperglycemia due to an absolute or relative lack of insulin and/or insulin resistance (Mohamed *et al*., 2012). Anderson *et al*., (2001) have suggested that approximately 11% of patients with diabetes suffer from major depression. Depression is a state of low mood and aversion to activity. It has been linked to hyperglycemia and poor blood glucose control (Lustman *et al*., 2000). People with diabetes have two-fold increased risks of having depression as compared with people without diabetes (Egede, 2004). Diabetes is more common in people who had suffered long-term sorrow as depression enhances the risk of diabetes. Whether depression leads to diabetes or it is a complication of diabetes is still not clear (Lustman and Clouse, 2007). However, it is likely to be bi-directional involving both behavioral and neuro-hormonal factors (Castillo-Quan *et al*., 2010).

Both depression and diabetes are known to activate the hypothalamic-pituitary-adrenal axis (HPA), which may contribute in worsening the metabolic control of Type 2 diabetes and thereby inducing a higher prevalence of diabetic complications (Chiodini *et al*., 2007).

Research studies support an association between serotonergic function, depressed mood, chronic glycemic control and dysregulation of HPA axis (Van den Akker *et al*., 2004). Serotonin (5-Hydroxytryptamine, 5-HT) is a fundamental neuromodulator in both vertebrate and invertebrate nervous system, and has been implicated in the etiology of many diseases particularly mental illness such as anxiety and depression (Haleem, 2009). Dopamine also a neurotransmitter plays an important role in fear and emotional response to stress (Bratcher *et al*., 2005). The serotonergic system is known to inhibit dopamine neurotransmission at the level of the origin of dopamine system in the midbrain as well as in the terminal regions of the brain (Samad *et al*., 2007).

The hypothesis that anti-diabetic drugs may lead to behavioral deficit and neurochemical changes in the brain was tested in rat models in the present study. The drug administered was Glimepiride (Trade name: Amaryl), one of the third generation sulfonylurea compound, used in the treatment of type 2 diabetes. Its mechanism of action is its ability to enhance insulin release from β-cells of pancreas (Ammara *et al*., 2006). It improves both first and second phase of insulin secretion and acts via extra-pancreatic mechanism (Shukla *et al*., 2004; Mori *et al*., 2008).

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The present study was conducted on rats to monitor behavior in monitor behavior in models of anxiety/ depression and alteration in brain serotonin and dopamine following acute administration of glimepiride at high doses.

MATERIAL AND METHODS

Animals

Twenty-four locally bred male Albino-Wistar rats weighing 200-250gm purchased from DUHS animal house were used in the study. All animals were housed individually placed in an environmentally controlled room at room temperature (25±2°C) under a 12:12 hr light/dark cycle. The animals had free access to standard rodent diet and tap water. For acclimatization the animals were kept for 3 days. All experiments were conducted according to the protocol approved by Institutional Animal Ethics Committee (IAEC).

Drug and doses

Glimepiride (Glenmark Generics limited, India) was freshly prepared in slightly warm saline (0.9% NaCl) and was injected intra-peritoneally (i.p) at the doses of 2.5mg/kg, 5.0mg/kg and 10.0mg/kg respectively. Saline was injected to control animals at the dose of 1.0ml/kg.

Experimental protocol

Twenty-four animals were randomly divided into four groups each containing six animals. The groups were labeled as: (i) Saline- (ii) Glimepiride (2.5mg/kg)- (iii) Glimepiride (5.0mg/kg)- and (iv) Glimepiride (10.0mg/kg) injected animals. Animals were injected with saline or respective doses of Glimepiride for two weeks. Behavioral activities in Skinner's box, open field and elevated plus maze of each rat were monitored 20, 35, and 45 minutes post injection respectively. Animals were then decapitated one-hour post injection to collect brain samples. Samples were kept at -70ºC until neurochemical analysis by High Performance Liquid Chromatography with Electrochemical detection (HPLC-EC) was performed (Ikram *et al*, 2011).

Behavioral procedures

Skinner's Box Activity

Motor – related effects of the drugs were monitored in a Perspex activity cage the "Skinner's box" (A transparent rectangular box with dimension 26x26x26 cm) with saw dust covered floor. 15 minutes before monitoring the activity animal was placed in the box for habituation. The activity was monitored as counts of cage crossings /10 minutes starting 20 minutes post injection (Ikram *et al*., 2007; Mirza *et al*., 2013).

Open field activity

The open field apparatus used is a box with square area of 76x76cm with walls 42cm high and the floor divided by lines into 25 equal squares. The animal was placed in the center square of the open field. Activity was recorded as number of square crossed with all four paws for 5min (Ikram and Haleem, 2011; Haleem *et al*., 2013; Haleem and Ikram, 2013).

Elevated plus maze activity

The elevated plus maze apparatus used as an animal model of anxiety, consisted of four arms in which two were open and two were closed. The arms were of identical length (50cm) and width (10cm). The arms were joined by central area of $5cm^2$. The maze was elevated from the floor at a height of 60cm. Activity was noted as entries and time spent in open arm for 5 minutes (Ikram *et al*., 2014).

Collection of brain samples

Animals were killed 1hr post injection. The skull plates were cut and membrane covering the brain was removed with the help of fine forceps. Using spatula, brain was taken out and washed with ice-cold saline. The collected brains were immediately stored at -70ºC for the determination of dopamine and serotonin metabolism using HPLC-EC (Haleem and Khan, 2003)

HPLC-EC analysis of dopamine (DA), 5HT and their Metabolites

Biogenic amines and their metabolites were extracted with 150µL of perchloric acid (70%) from brain tissue punches (<250 µ g) using a simple one-step sample preparation method. A 5- µm (particle size) ODS column (4.0mm i.d and 250 mm length) was used. Mobile phase comprising methanol (14%), octyl sodium sulphate (0.023%) and EDTA (0.0035%) in 0.1molL-1 Phosphate buffer of pH 2.9 was passed through the column at a constant flow rate (1.0 ml min-1) with the help of a water 510 HPLC pump (Waters Corporation, USA). Brain samples were homogenized by using electrical homogenizer and subjected to centrifugation at 6000 rpm for 20 minutes at 4ºC. Supernatant was separated and injected to HPLC-EC for neurochemical analysis. Electrochemical detection was achieved on a shimadzu L-EC 6A detector (Shimadzu, Japan) at an operation potential of +0.8V (Ikram *et al*., 2012).

STATISTICAL ANALYSIS

The data is presented as means ± SD. Analysis of the data was done by one- way ANOVA. Post-hoc comparisons were done by Newman-Keuls test. Values of $p<0.05$ were considered statistically significant

RESULTS

Fig. 1 shows the results of effects of different doses of Glimepiride administration (2.5, 5.0 & 10.0mg/kg) on activities of rats in Skinner's box and open field as analyzed by one-way ANOVA. The activities were monitored 20 and 35 minutes for 10 and 5 minutes post

injection, respectively. The activities in Skinner's box (F=0.432; df=3, 20) and Open field (F=0.145; df=3, 20) were not significant. The observations indicate that the drug had no CNS stimula-tory effects in familiar- as well as novel environment.

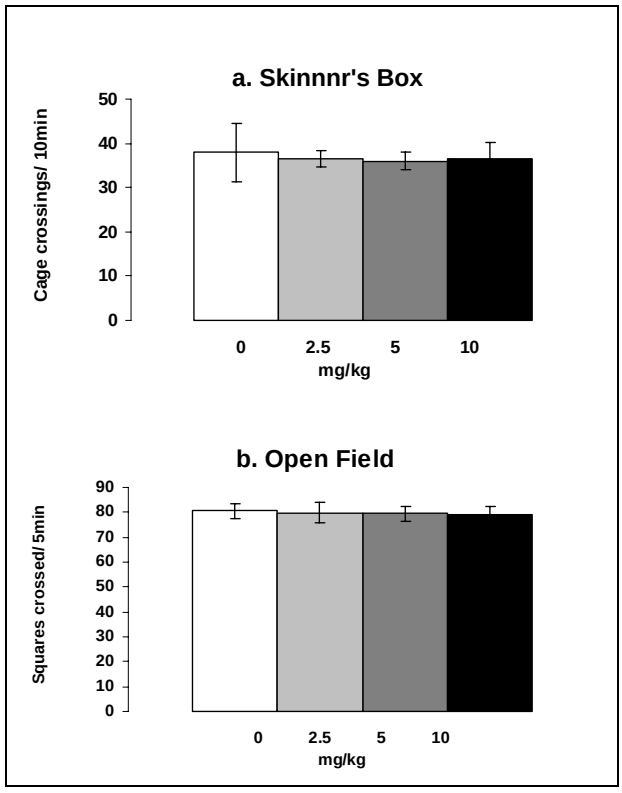

Fig. 1: Effects of different doses (2.5, 5.0 & 10mg/kg) of Glimepiride on (a) Skinner's box and (b) Open field activities 20 & 35 min post injection respectively. Values are mean ± SD (n=6). Differences were not significant following one -way ANOVA.

Fig. 2 shows effects of glimipride on elevated plus maze activity. Analysis of the data by one way ANOVA showed that effects of glimipride on number of entries in open arm (F=14.61; df=3, 20; p<0.05) and time spent in the open arm (F=17.34; df=3,20; p<0.05) were all significant, as monitored 45 minutes post injection for 5 minutes. Post hoc analysis by Newman-Keuls test showed that the time spent in open arm decreased significantly at all the three doses, but the decrease at higher dose (10.0mg/kg) was much more (p<0.01) as compared to low and moderate doses (2.5 and 5.0mg/kg).

Fig. 3 shows effects of glimipride on dopamine and metabolites. Analysis of the data by one-way ANOVA showed that the effects of glimipride on dopamine (F=0.537; df=3, 20) and DOPAC (F=1.62; df=3, 20) were nonsignificant. While a significant decrease of HVA (F= 7.48; df=3, 20; p<0.05) was observed. Post hoc analysis by Newman Keuls test showed decreased (p<0.05) HVA levels at all three doses.

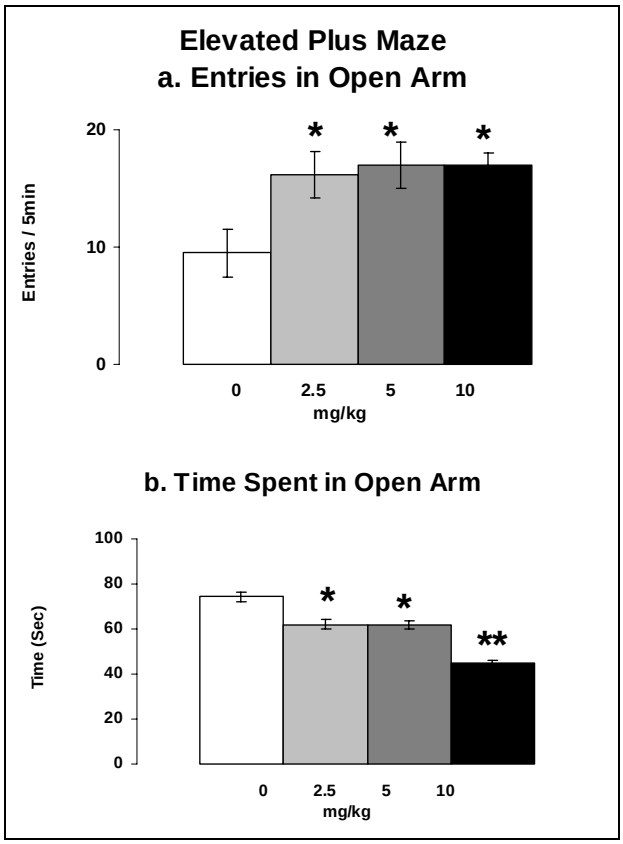

Fig. 2: Effects of different doses (2.5, 5.0 & 10mg/kg) of Glimepiride on (a) numbers of entries and (b) time spent in open arms as monitored 45 min post injection. Values are means ± SD (n=6). Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from saline injected controls following one-way ANOVA

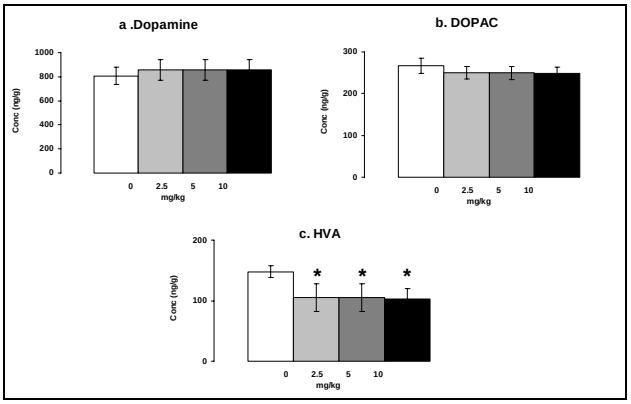

Fig. 3: Effects of different doses (2.5, 5.0&10.0mg/kg) of Glimepiride on (a) dopamine and metabolites; (b) DOPAC and (c) HVA. Values are means ±SD (n=6). No significant difference in DA and DOPAC. Significant difference in HVA from saline injected controls by Newman-Keuls test *p<0.05 following one-way ANOVA

Fig. 4 shows effects of glimipride on 5-HT and metabolite. Data on effects of glimipride, as analyzed by one-way ANOVA, showed that the drug significantly

altered 5-HT (F=0.02; df=3, 44; p<0.05) levels. Post hoc analysis by Newman keuls test showed decreased (p<0.05) 5-HT levels at moderate and high doses (5.0- and 10.0 mg/kg) only. Analysis of the data on 5-HIAA levels, by one-way ANOVA showed significant effects of the drug on 5-HIAA levels (F=10.23; df=3, 20; p<0.05). Post hoc analysis by Newman keuls test showed decreased (p<0.05) 5-HIAA levels at all three doses of the drug.

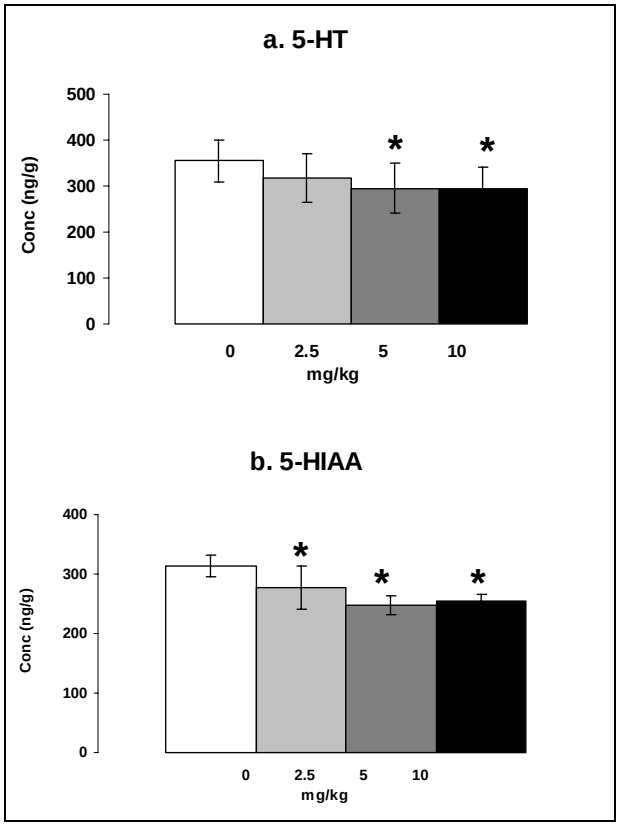

Fig. 4: Effects of different doses (2.5, 5.0 & 10.0mg/kg) of Glimepiride on (a) 5-HT and its metabolite; (b) 5-HIAA. Values are means ±SD (n=6). Significant differences by Newman-Keuls test: *p<0.05 from saline injected controls following one-way ANOVA

DISCUSSION

In rodents, one of the most important components of locomotion is spontaneous activity in open field. Locomotive responses to novelty are an animal index of exploration/ anxiety. The individual differences in locomotive activity and reactivity to novelty are related to anxiety- and depression -like responsiveness in rats (Cagni *et al*., 2008). The present study utilized Skinner's box for locomotive activity, and open field for the exploration of novel environment.

The elevated plus maze model is based on rodent's aversion of open spaces. Anxiogenic drugs reduce time spent on open arms while anxiolytic drugs increase time spent on open arms of the elevated plus maze (Ribas *et al*., 2008). The Present study suggests that the drug at particularly higher dose has anxiogenic like effects. Symptoms of depression and anxiety are regarded as significant risk factors for onset of type 2 diabetes independent of other established risk factors for diabetes (Engum, 2007). One major factor, which can affect therapeutic profile of anti-diabetics, seems to be their inability to treat depression or properly project depression (Rotella and Mannucci, 2012).

Diabetes causes a decrease in limbic dopamine. Murzi *et al* (1996) have reported decreased DOPAC basal levels as well as decreased amphetamine-induced dopamine release in diabetic rats. The present study on the contrary to the previous study observed decrease HVA levels. This finding could be due to a higher dose of glimepiride (10mg/kg/b/w) injected to the rats. The present study shows a relationship between glimepiride administration and decreased monoamine metabolism, especially that of serotonin. Since serotonergic deficits are related to depression (Haleem, 2011). The present results suggest that glimepiride induced decrease of brain serotonin may elicit depression in diabetic patients treated with the drug.

A decrease in 5-HT metabolism is associated with depression (Stockmeier, 2003). The present study observed a decrease in 5-HT, as well as in its metabolite 5- HIAA. Thus present results suggest that short-term depression-like behavior as observed in the diabetic patients may be due to the decreased availability of 5-HT. Other studies have also have reported poorer clinical and functional outcomes in diabetics suffering from depression, despite all possible treatment efforts (Koike *et al*., 2002). The therapeutic effects of antidepressants are associated with 5-HT system (Artigas, 2013) and a deficiency of serotonin precipitates depression. In the present study Glimepiride has shown to decrease 5-HT metabolism (fig. 4). We therefore suggest that mild-to-moderate or severe depressive episodes in diabetic patients treated with glimepiride, might be observed which may impair therapeutic profile of the anti-diabetic drug.

However, few points have to be considered in regard to the present study. The drug administered at 10mg/kg b/w was considered lethally hypoglycemic dose for rats according to study by (Yadav *et al*., 2010). The study observed the dose of 5.0mg/kg was safe and potent.

CONCLUSION

In conclusion we report that the antidiabetic drug glimepiride induces neurochemical deficits and depression-like behavior in rat models. We suggest that patients treated with glimipride should be monitored for glimipride-induced deficits of serotonin and related conditions.

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