

Behavioral Effects of Anti-Diabetic Drugs on Normal Rats: A Dose Related Study

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Abstract: Background: Patients with diabetes are at increased risk of developing depression. The high prevalence of depression in diabetes could also be due to use of anti-diabetic drugs. The aim of the present study was to monitor changes in locomotor, exploratory, and anxiety behavior if any, in normal rats treated with two different kinds of anti-diabetic drugs at various doses.

Methodology: Forty Eight Albino-Wistar rats weighing 180 – 200 gms were used. Experiments were conducted according to the protocol approved by Institutional Animal Ethics Committee (IAEC). Glimperide (Trade name; Amaryl) and Pioglitazone (Trade name; Actos or Zolid) were injected intra-peritoneal at doses of 2.5 5.0 and 10.0mg/kg to the respective animals. Behavioral activities of each rat in Skinner's box, open field and in open arm of elevated plus maze were monitored 20, 35, and 45 minutes post injection respectively. Data were analyzed by one- way ANOVA. Post-hoc comparison was done by Newman-Keuls test. Value of $P < 0.05$ were considered statistically significant.

Results: Glimperide did not alter Skinner's box and open field activities but decreased number of entries and time spent in open arm of elevated plus maze ($p < 0.05$). Pioglitazone decreased open field but not Skinner's box activity. Number of entries and time spent in the open arm of the elevated plus maze also decreased.

Conclusion: The results are relevant that Glimperide as well as Pioglitazone elicit anxiety like behavior and may be a cause of depression observed in patients on long term therapy of these drugs. Pioglitazone but not Glimperide also has sedative effects.

Key Words: Diabetes, Depression, Glimperide, Pioglitazone, Behavioral activities.

INTRODUCTION

Diabetes is a chronic metabolic disorder affecting 170 million individuals globally [1]. A study reported that the prevalence of depression in diabetic subjects is higher than that in the general population [2]. The high prevalence of depression in diabetes may be due to social, cultural or economic factors [3]. There is evidence that patients with diabetes are at increased risk of developing depression although a bidirectional relationship might also exist [4]. A number of clinical studies support the relationship between developing depression in diabetic patients and the increase in morbidity and mortality of these patients [4], but little is known about the neurochemical mechanisms that would constitute its biological basis.

The hypothesis that anti-diabetic drugs may produce depression like behavior and induce behavioral deficits was tested in rat models in the present study.

Commonly used anti-diabetic drugs such as Glimperide (Trade name; Amaryl) and Pioglitazone (Trade name; Actos or Zolid) were administered at therapeutic doses to monitor acute effects on behavior of rats. Both the drugs are used in the treatment of type 2 diabetes mellitus.

Glimperide is a sulfonylurea compound with molecular weight 490.62 and molecular formula $C_{24}H_{34}N_4O_5S$. The hypoglycemic activity of glimepiride is relied on its ability to enhance insulin release from β - cells of pancreas and acts via extra-pancreatic mechanism [5].

Pioglitazone HCl is a member of Thiazolinediones group of drugs. It has molecular weight 392.90 and molecular formula $C_{19}H_{20}N_2O_3S.HCl$. It is also known as "insulin sensitizer". Pioglitazone is chemically nor functionally related to sulfonylureas. It selectively stimulates nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-gamma). Activation of PPAR-gamma receptors regulates the transcription of insulin-responsive genes involved in the control of glucose production, transport and utilization [6].

Over the years animal models have been used for investigations into the aetiology and treatment of various human disorders [7]. There is a wide range of animal models showing changes in activity in a familiar and novel environment [8]. Also the use of Elevated Plus Maze as an animal model of anxiety for rodents has been validated to assess the anti-anxiety effects of pharmacological agents [9].

Thus the aim of the present study was to monitor changes in locomotor, exploratory, and anxiety behavior if any, in normal rats treated with two different kinds of anti-diabetic drugs at various doses.

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MATERIAL AND METHODS

Animals

Forty Eight locally bred Albino-Wistar rats weighing 130-230 gms purchased from DUHS animal house were used in the study. All animals were housed individually in Perspex cages. The animals had free access to standard rodent diet and tap water. They were placed in an environmentally controlled room at room temperature ($25 \pm 2^\circ\text{C}$) under a 12:12 hr light/dark cycle (lights on at 6:00 hr). 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 Soses

Glimepiride and Pioglitazone were purchased from Glenmark Generics limited and Dr Reddy's Pharmaceutical Co Ltd, India respectively. The drugs were freshly prepared before starting the experiment. The two drugs were dissolved separately in slightly warm saline (0.9% NaCl) and injected intra-peritoneal (i.p) at doses of 2.5mg/kg, 5.0mg/kg and 10.0mg/kg to the respective animals. Saline (0.9% NaCl solution; 1ml/kg) was injected to control animals.

Experimental Protocol

Experiments on two drugs were performed separately.

(i) Glimepiride (ii) Pioglitazone

Twenty four animals were randomly divided into four groups each containing six animals. The groups were labeled as: (i) saline injected; control. (ii) Glimepiride (2.5mg/kg) (iii) Glimepiride (5.0mg/kg) and (iv) Glimepiride (10.0mg/kg). Animals were injected with saline (0.9% NaCl solution) or respective doses of Glimepiride. Behavioral activities in Skinner's box, open field [10] and elevated plus maze [11-12] of each rat were monitored 20, 35, and 45 minutes post injection respectively.

Behavioral Procedures

Dose –dependent effect of glimepiride on motor activity in a familiar environment

Skinner's Box

Motor – related effects of the drugs were monitored in a Perspex activity cage the "Skinner's box" (A transparent rectangular box with dimension 26x26x26 cms) 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 [10]

Dose-dependent effect of glimepiride on exploratory activity in a novel environment

Open Field Activity

The open field apparatus used is a box with square area of 76x76 cms with walls 42 cms 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 5 minutes [10]

Dose-dependent effect of glimepiride on anxiogenic and anxiolytic activity.

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 (50cms) and width (10cms). The arms were joined by central area of 5 cms². The maze was elevated from the floor at a height of 60 cms. Activity was noted as entries and time spent in open arm for 5 minutes respectively [11-12].

(ii) Pioglitazone injected: Twenty four animals were randomly divided into four equal groups. Control was injected with saline at 1ml/kg body weight. While Pioglitazone test groups were injected i.p at doses of 2.5, 5.0 and 10 mg/ml/kg respectively. The protocol for behavioral activities was the same as mentioned for the drug Glimepiride.

Statistical Analysis

The results are presented as means and \pm SD. Data were analyzed by one- way ANOVA. Post-hoc comparison was done by Newman –Keuls test Values of p less than 0.05 were considered statistically significant.

RESULTS

Effects of different doses of Glimepiride administration (2.5, 5.0 & 10.0 mg/kg) on activities of rats in Skinner's box and Open field are shown in Fig. (1). Activities were monitored 20 and 35 minutes post injection for 10 and 5 minutes respectively. Effects of Glimepiride on Skinners box activity ($F=0.432$; $df=3, 20$; $p>0.05$) and activities in Open field ($F= 0.145$; $df= 3, 20$; $p>0.05$) were not significant. The results therefore indicate that the drug had no CNS stimulatory like effects in familiar as well as in novel environment.

Fig. (2) Shows effects of different doses (2.5, 5.0 & 10.0 mg/kg) of glimepiride on (a) number of entries and (b) time spent in the open arm of Elevated Plus Maze as monitored 45 minutes post injection for 5 minutes. Effects on number of entries ($F=14.615$; $df=3, 20$; $p<0.05$) and time spent ($F=17.340$; $df= 3, 20$; $p<0.05$) were all significant. Administration of Glimepiride increased entries in open arm ($p<0.05$) at all three doses as compared to saline injected control. Time spent in open arm decreased ($p<0.05$) at all the

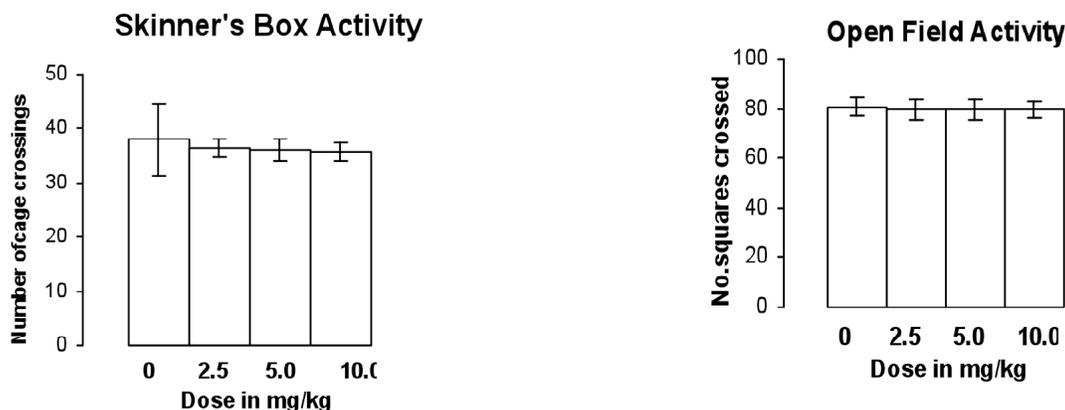


Fig. (1). Effects of different doses (2.5, 5.0&10.0mg/kg) of Glimpiride on Skinner's box and Open field Activity 20 & 35 mins post injection respectively. Values are means \pm SD (n=6). Differences in Skinner's box and open field activity were not significant following one-way ANOVA

Elevated Plus Maze Activity

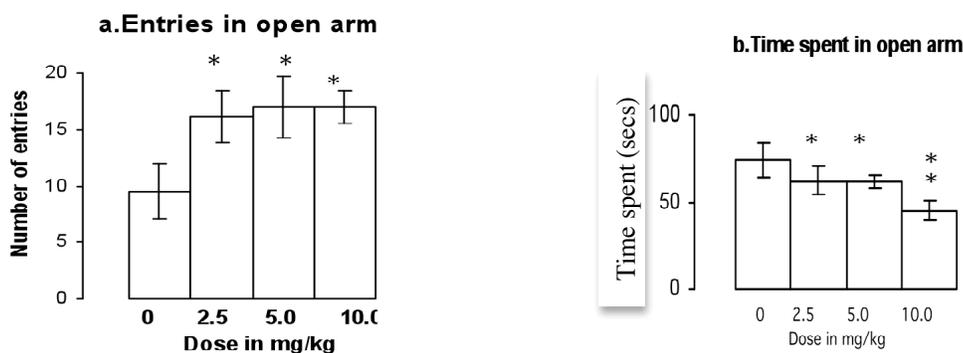


Fig. (2). Effects of different doses (2.5, 5.0&10.0mg/kg) of Glimpiride on number of (a) entries and (b) time spent in open arm as monitored 45 mins post injection. Values are means \pm SD (n=6). Significant differences by Newman-Keuls test: * p <0.05; ** p <0.01 from saline injected controls following one-way ANOVA.

three doses, but the decrease at higher dose (10.0mg/kg) was much more as compared to low and moderate doses (2.5 and 5.0 mg/kg), p <0.01. The results therefore suggest that the drug at particularly higher dose has anxiogenic like effects.

Effects of different doses (2.5, 5.0 & 10.0 mg/ kg) of Pioglitazone on Skinner's box and Open field activities are shown in Fig. (3). Activities were monitored 20 and 35 minutes post injection for 10 and 5 minutes respectively. Effects were not significant ($F=1.397$; $df=3, 20$; $p>0.01$) on Skinner's box but significant in Open field. ($F=5.758$; $df=3, 20$; $p<0.05$).

Post-hoc test showed that the drug decreased activity at all the three doses compared to saline injected controls in open field. The results suggest that the anti-diabetic drug Pioglitazone unlike Glimpiride has CNS depressant like effect.

Fig. (4). Shows effects of Pioglitazone administration at doses (2.5, 5.0 and 10.0mg/kg) on (a) number of entries and

(b) Time spent in the open arm of Elevated Plus Maze as monitored 45 minutes post injection for 5 minutes. Effects on number of entries ($F=11.338$; $df=3, 20$; $p<0.05$) and time spent ($F=4.724$; $df= 3, 20$; $p<0.05$) were significant at all the three doses as compared to saline injected controls. Post hoc test showed that the drug decreased number of entries as well as time spent in open arm at all the three doses used. The results suggests that Pioglitazone similar to Glimpiride produces anxiogenic like effects.

DISCUSSION

Diabetes is a major threat to global public health that is rapidly getting worse, and the biggest impact is on adults of working age in developing countries [13]. Diabetes itself causes stress and stress is the major precipitating factor in the onset of depression which is supported by clinical observation [14].

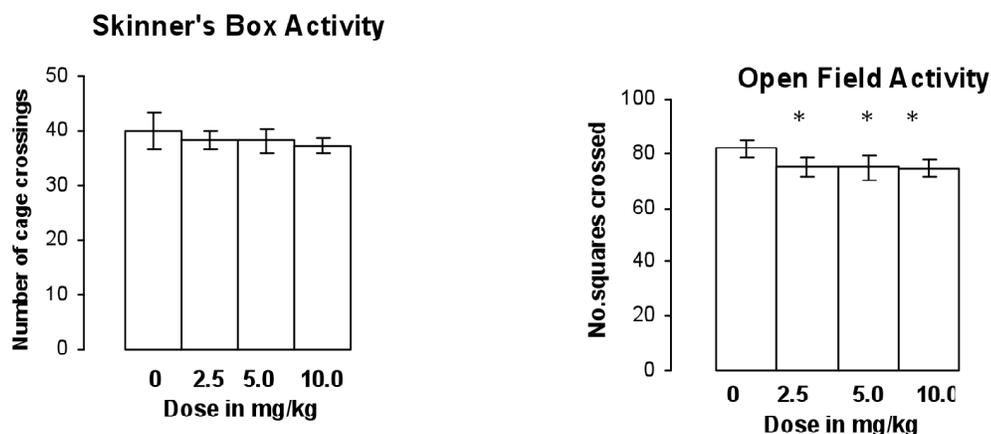


Fig. (3). Effects of different doses (2.5, 5.0 & 10.0 mg/kg) of Pioglitazone on Skinner's box and Open field activity as monitored 20 and 35 mins post injection respectively. Values are means \pm SD (n=6). Significant differences by Newman-Keuls test: * $p < 0.05$ from saline injected controls following one-way ANOVA.

Elevated Plus Maze Activity

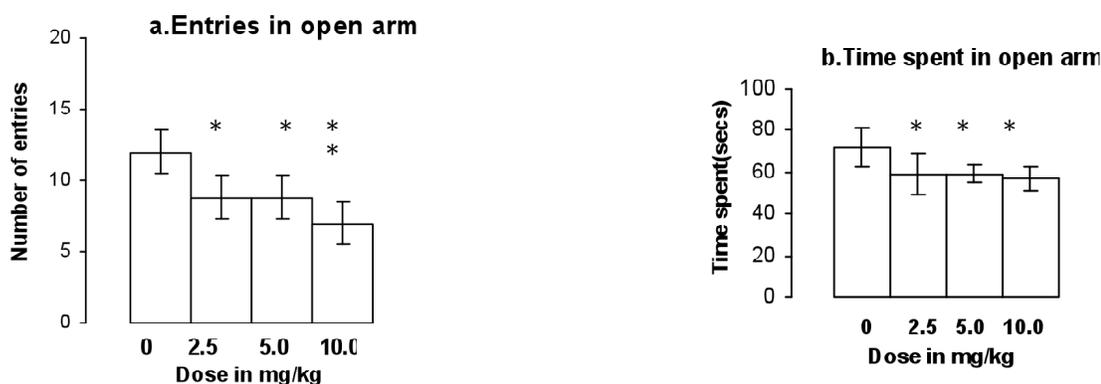


Fig. (4). Effects of different doses (2.5, 5.0 & 10.0 mg/kg) of Pioglitazone on (a) number of entries and (b) time spent in open arm as monitored 45 mins post injection. Values are means \pm SD (n=6). Significant differences by Newman-Keuls test: * $p < 0.05$; ** $p < 0.01$ from saline injected controls following one-way ANOVA.

But, however, little information is available on the behavioral effects produced by the use of anti-diabetic drugs in diabetic patients.

The use of sulfonylureas in diabetic patients has several problematic issues like hypoglycemia, nausea, stomach upset, and weight gain [15]. Thiazolidinediones compounds can cause retention of fluid leading to ankle swelling, breathlessness and a gain in weight [15]. But the changes in central nervous system (CNS) and therefore in behavioral activity produced by use of these drugs has never been documented.

Our study used Rat models to observe the changes in behavioral activities brought by use of two different glucose lowering agents; Glimperide (Sulfonyurea) and Pioglitazone (Thiazolidinediones). Some studies have used for assessment of locomotor activity, the Skinner's box and for exploration in Novel environment, the Open field [16-17]. Our study utilized these two behavioral activities.

Locomotor activity refers to the movement from one location to another. In rodents, one of the most important components of exploration a prominent activity of the rats repertoire of spontaneous activity, is locomotion. The locomotor activity and exploration are involved in many behavioral and physiological functions. Locomotor responses to novelty is an animal index of exploration /anxiety. Therefore this animal model is widely used to investigate whether individual differences in locomotor activity and reactivity to novelty are related to anxiety –and depression-like responsiveness in rats [18].

The present results on the behavioral effects of Glimperide and Pioglitazone on rats are relevant that at different doses of Glimperide there was no change in activities in Skinner's box as well as in Open field (Fig. 1). While Pioglitazone decreased activity in Open field ($p < 0.05$; Fig. 3). The results show that unlike Glimperide, Pioglitazone has some anxiogenic like effect. It is important to note that Pioglitazone unlike Glimperide decreased motor acti-

vity in novel environment. Our study has therefore observed impaired motor co-ordination as effect of Pioglitazone.

The elevated plus maze is a rodent model of anxiety test that is used as a screening test for putative anxiolytic / anxiogenic compounds and as a general research tool in neurobiological anxiety research [19]. The model is based on rodent's aversion of open spaces. This aversion leads to behavior which involves avoidance of open areas by confining movements to enclosed spaces or to the edges of a bounded space. In elevated maze test this translates into a restriction of movements to the enclosed arm [20]. The study by Ribas et al., 2008 have reported that the time spent in the open arms was used as an anxiety index [21]. Anxious animals will spend more time in the closed arms than less anxious animals [22].

The results on behavior of rats on elevated plus maze, show that at all the three doses of Glimpiride there is significant increase in entries in open arms ($p < 0.05$, Fig. 2), while the time spent in open arms decreased significantly at all the three doses ($p < 0.05$, Fig. 2), but the decrease at higher dose (10.0mg/kg) was much more as compared to low and moderate doses (2.5 and 5.0mg/kg). The result therefore suggests that Glimpiride at particularly higher dose has anxiogenic like effects.

Anxiogenic drugs reduce time spent on the open arm and anxiolytic drugs increase the time spent on the open arm of the elevated plus maze [9]. The number of entries as well as time spent on open arms decreased significantly ($p < 0.05$; Fig. 4) at all the three doses of Pioglitazone. This drug is therefore anxiogenic.

CONCLUSION

In clinical trials with Glimpiride and Pioglitazone depression was not seen as either common or rare side effects, but that does not mean that these anti-diabetic drugs cannot cause depression in an individual. The results of the present study indicate that Glimpiride as well as Pioglitazone have anxiogenic like effects, but as this was an acute study a chronic study is needed to prove the drugs anxiogenic effects.

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