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Original Article

Prolonged hyperglycemia decreased the adverse respiratory effects of benzodiazepines in rats

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Abstract

Introduction: The incidence of diabetes is increasing along with its associated respiratory disorders, sleep disturbance and mental health problems. Despite the adverse effects of benzodiazepine receptor agonists (BZRAs) on the respiratory system function, they remain the most commonly used medications for the management of anxiety and sleep disorders. The aim of this study was to investigate whether chronic hyperglycemia increases the adverse respiratory effects of BZRAs.

Methods: The experiments were carried out in male Wistar rats that were randomly allocated into six experimental groups. Hyperglycemia was induced by injecting 35mg/kg streptozotocin (STZ). We recorded breathing of conscious animals using whole-body plethysmography at the onset of the experiment and three weeks after diabetes induction. Animals were subjected to intraperitoneal injection of midazolam (0.75mg/kg) and diazepam (1mg/kg) 15min prior to the second respiratory recording.

Results: Analysis of respiratory dynamics revealed an alteration in breathing pattern in intact animals following the anxiolytic dose of benzodiazepines, which was associated with an increase in respiration rate and variability and decrease in the irregularity of the respiratory rhythm. Meanwhile, these effects were significantly decreased in hyperglycemic animals.

Conclusion: Our results demonstrate that STZ-induced hyperglycemic rats exhibited decreased adverse respiratory effects of BZRAs. It seems that hyperglycemia induced an impairment in benzodiazepine receptors response to the BZRAs.

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Keywords:
Hyperglycemia;
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Introduction

Diabetes mellitus is a chronic endocrine disorder and one of the leading causes of morbidity and mortality worldwide. A wide range of complications that accompany diabetes, including serious mental health problems, respiratory disorders and sleep disorders (Weisbrod et al., 2005; Ducat et al., 2014; Lecube et al., 2015; De Santi et al., 2017; Robinson et al., 2018). Previous studies have reported a high prevalence of psychiatric disorders such as anxiety, depression and sleep disorders in diabetic patients. Subjects with diabetes, mental health concerns and sleep disorders have increased functional

impairment, daytime tiredness/sleepiness and decreased quality of life (Skomro et al., 2001; Einhorn et al., 2007; Reutrakul and Mokhlesi, 2017; Robinson et al., 2018).

Sedatives and hypnotics have been prescribed to treat and manage of sleep disorders and mental health problems. Benzodiazepine receptor agonists (BZRAs) are among the most commonly used drugs for treating subjects with insomnia, including those with comorbid conditions like diabetes (Reutrakul and Mokhlesi, 2017). Benzodiazepine receptors are expressed in the membrane of neuronal cells throughout the nervous system. By binding to these receptors, it increases their permeability for chloride ions through GABA channels and thus, decreases the excitability of neurons in the brain and sedation is achieved (Young and Chu, 1990; Kaila, 1994). However, the most concerning side effect associated with benzodiazepines use is respiratory depression, which may aggravate respiratory disorders such as apnea (Montplaisir et al., 2003) in patients with diabetes. Many studies have investigated the function of the GABAergic system and the effect of GABA receptors agonist on central nervous system (CNS) in diabetes (Ramanathan et al., 1998; Antony et al., 2010; Yasin Wayhs et al., 2015). Evidences from preclinical and clinical studies indicate that the GABAergic system plays a role in the pathophysiology of diabetic encephalopathy-related depression (Yasin Wayhs et al., 2015). A recent study demonstrated an excitatory/inhibitory neurotransmitter imbalance in the CNS of diabetic patients and GABA levels were significantly lower within these patients (Petrou et al., 2012). Ramanathan et al. (1998) have demonstrated that the anxiolytic effect of diazepam was decreased in streptozotocin (STZ)-induced diabetic rats. However, evidence on the effect of BZRAs use on respiratory disorders such as apnea in diabetic patients is limited. It is a very important clinical and public health problem because anxiety and insomnia are prevalent in diabetic patients and BZRAs are broadly prescribed. In the present study, we investigated whether chronic hyperglycemia (HG) induced by STZ can alter the respiratory effects of the two most common BZRAs diazepam and midazolam in rats.

Materials and methods

The experiments were carried out on male Wistar rats, weighing 180–220g that were housed in standard cages with food and water *ad libitum*. The room temperature was maintained at 22±2 °C with a light:dark cycle of 12:12 h. All animal experiment protocols were approved by the “Ethical Committee of Arak University of Medical Sciences” (IR.ARAKMU.REC.1398.164).

Drugs

STZ (Sigma, St. Louis, MO, USA) freshly dissolved in 0.1M citrate buffer (pH 4.5). The anxiolytic dose of midazolam (0.75mg/kg; Exir Pharmaceutical Co., Iran) and diazepam (1.0ml/kg; Caspian Tamin Pharmaceutical Company) were administered intraperitoneally (ip).

Induction of HG

HG was induced by a single intraperitoneal injection of 35mg/kg STZ which was freshly. Four days later, the presence of HG was confirmed by measuring serum glucose concentrations. The rats with blood glucose ≥200mg/dl were considered to have HG. Animals with blood glucose higher than 350mg/dl were excluded from the experiment. In control group animals were subjected to saline injection.

Animal groups and experimental protocols

Rats were randomly divided into 6 groups, each comprising of 6–8 animals. Control, HG, midazolam (MDZ), diazepam (DIA), HG/MDZ and HG/DIA. Respiration was recorded at the beginning and end of the experiments in all animals, as follows: baseline respiratory recording was done in control, MDZ and DIA groups at the beginning of experiment before saline injection and 3 weeks later, in HG, HG/MDZ and HG/DIA groups base-line respiratory recording was performed before STZ injection and 3 weeks after HG confirmation. Animals in HG/MDZ, HG/DIA, MDZ and DIA groups were subjected to midazolam or diazepam injection 15min before second respiratory recording. Figure 1 illustrates the protocol of this experiment.

Respiratory recording

Respiration was recorded in conscious and unrestrained animal using whole-body plethysmograph (BIODAC-R172, Trita Wavegram Co., Iran). The apparatus included a cylindrical chamber (volume 2l) made of transparent Plexiglas

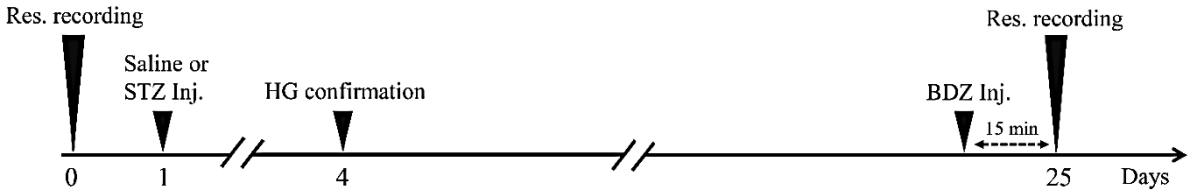

Fig.1. Timeline of the experimental protocol. Res: respiration, STZ: streptozotocin, Inj: injection, HG: hyperglycemia, BDZ: benzodiazepine.

with oppositely aligned inlet and outlet ports in each wall. Room air was constantly pumped into the cylinder at a controlled flow rate (4l/min) in order to prevent any alteration of CO2 in the plethysmograph. The chamber outlet was linked to a differential pressure transducer through a polyethylene tube (Pazhoohan et al., 2017). In order to accustom the rats to the recording cylinder animals were placed in the recording box 1h/day for 5 days before respiratory signal recording. On the experiment day, animals were gently placed into the recording box. Each recording consisted of a 10min initial recording period (acclimatization time), which was then followed by a 20min main recording period.

Analysis of breathing pattern

Respiratory signals were digitized at 1kHz and stored on a PC for offline analysis. Total time (20min) of main respiratory recording was use for breathing pattern analysis. Time-series of inter-breath interval (IBI) was calculated using a program written in MATLAB. Alteration in breathing pattern was quantified by calculating respiratory rate and the mean, coefficient of variation (CV), sample entropy (SampEn) and multiscale entropy (MSE) of IBI time series. Data of mean, CV, SampEn and MSE of IBI normalized as percentage of baseline values.

Sample entropy and MSE analysis

Sample entropy can be used to reveal the entropy of a biological signal, which is the negative natural logarithm of the conditional probability that two sequences similar form points remain similar at the next point within a tolerance (r), without allowing self-matches. Thus, a lower value of SampEn indicates more regularity in the time series (Richman and Moorman, 2000). MSE analysis estimates sample entropy of physiological signals at different time scales to determine if there are any correlations. MSE uses a coarse-graining process to generate many different time scales. After this process, the sample entropy is calculated for different scales and then plotted against a scale (Costa et al., 2002). Decreased entropy in a physiological time-series can be interpreted as decreased information processing or lower engagement the part of within a control system (Pincus and Goldberger, 1994).

Statistics

GraphPad Prism V6.07 (GraphPad Software, San Diego, CA) was used for statistical analysis of data. Data are presented as mean±SEM. Comparisons of data between groups were performed using one-way and two-way ANOVA analysis followed by Bonferroni's post hoc test.

Results

The differences in respiratory rate among experimental groups have been shown in Figure 2. The respiratory rate was significantly higher in the MDZ and DIA groups compared to the control group (P<0.05), while there was no significant difference in respiratory rate among HG/MDZ, HG/DIA and HG groups compared with control.

The mean IBI of experimental groups has been analyzed and demonstrated in Figure 3. Hyperglycemic animals were subjected to midazolam had significantly increased in the mean of IBI compared to MDZ injected animals (P<0.01).

Figure 4 demonstrates the CV of IBI time-series for all experimental groups. There was no significant difference in the CV of IBI between HG and control (P>0.05). However, the CV of IBI was significantly higher in the MDZ group compared to control (P<0.05) and HG/MDZ group (P<0.01).

Respiratory rhythm complexity, in which SampEn of IBI was used as an index of the irregularity, was significantly lower in the MDZ group in comparison with control and HG/MDZ (P<0.05, Fig 5a). However, there was no significant difference in the SampEn of IBI between control and HG. In addition, no significant difference was observed between

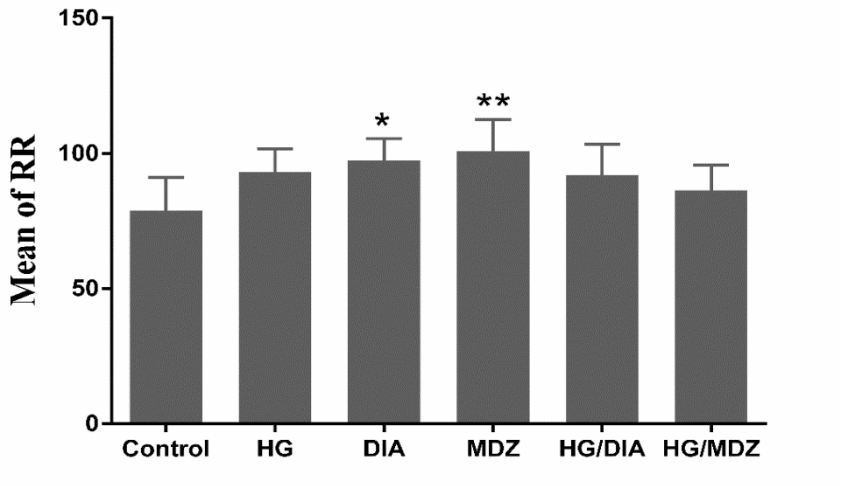

Fig.2. Mean values of respiratory rate (RR) was analyzed by one-way ANOVA with Bonferroni post-test. $^{*}P<0.05$ and $^{**}P<0.01$ compared to control. Data presented as mean±SEM (n=7). HG: hyperglycemia, DIA: diazepam, MDZ: midazolam.

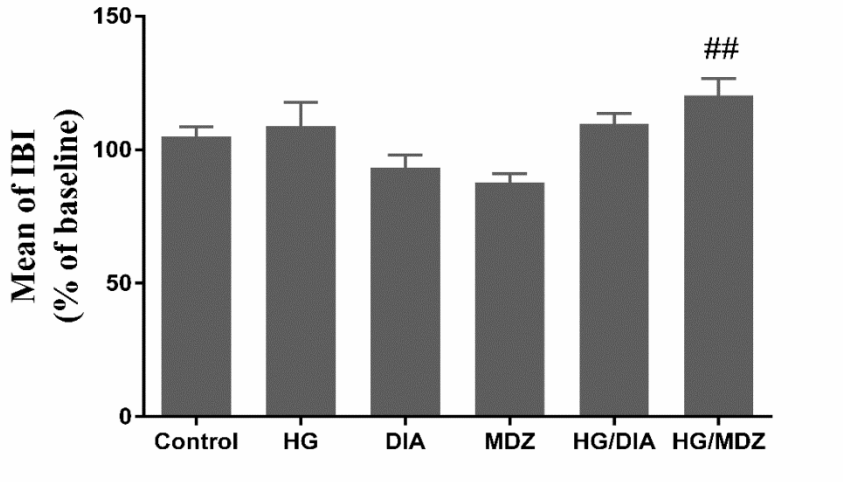

Fig.3. Mean of inter-breath interval (IBI) was analyzed by one-way ANOVA with Bonferroni post-test. $^{\#\#}P<0.01$ compared to MDZ. Data presented as mean±SEM (n=7). HG: hyperglycemia, DIA; diazepam, MDZ: midazolam.

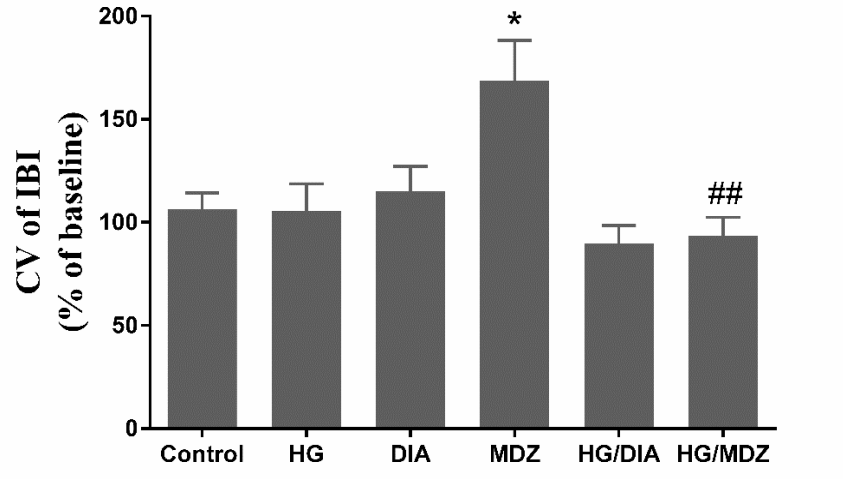

Fig.4. Coefficient of variation (CV) of IBI time-series was analyzed by one-way ANOVA with Bonferroni post-test. $^{*}P<0.05$ compared to control and $^{\#\#}P<0.01$ compared to MDZ. Data presented as mean±SEM (n=7). HG: hyperglycemia, DIA: diazepam, MDZ: midazolam.

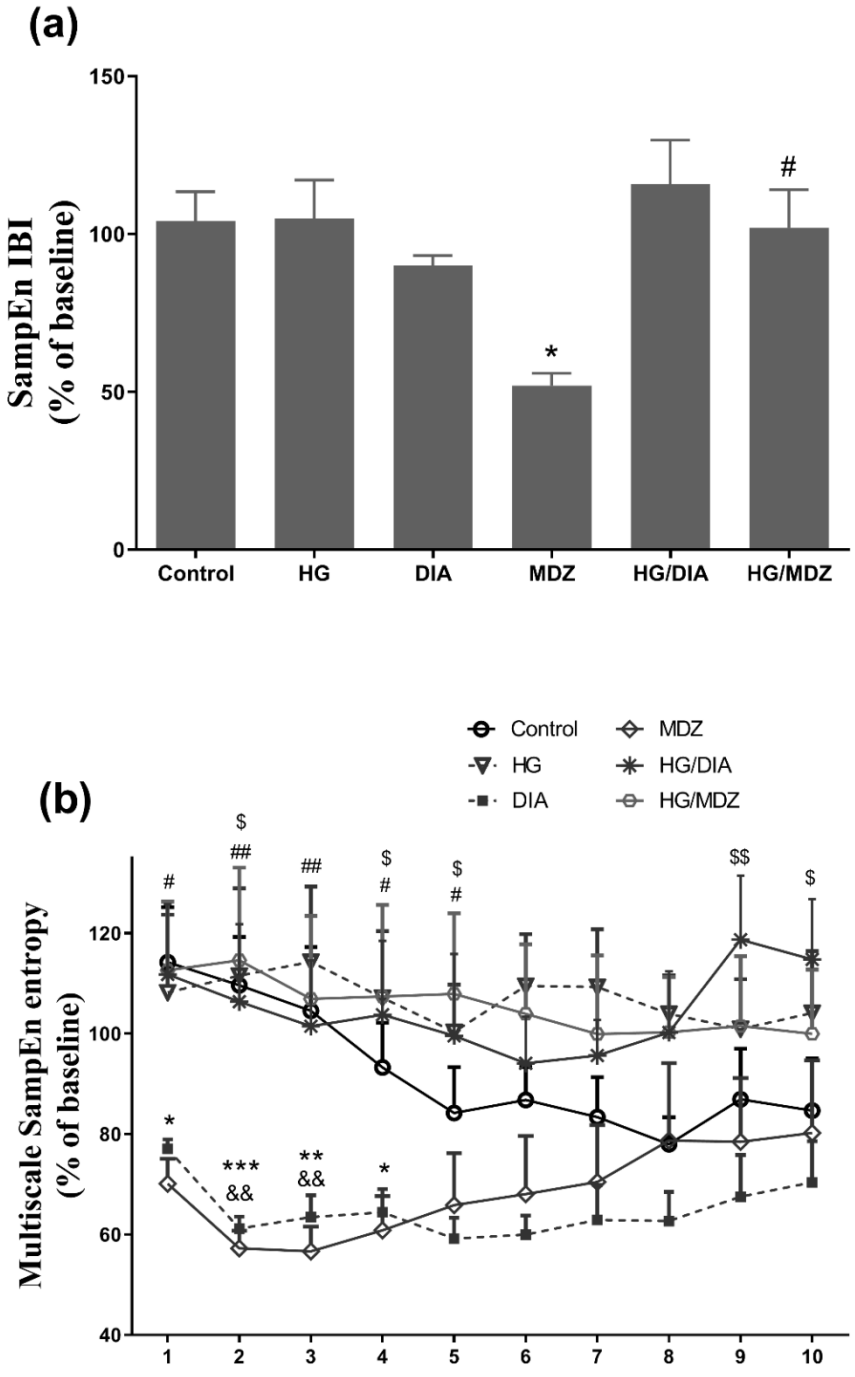

Fig.5. Differences of entropy among experimental groups. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ MDZ vs control; $^{\&\&}P<0.01$ DIZ vs control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ MDA/HG vs MDZ and $^{\$}P<0.05$, $^{\$\$}P<0.01$ DIA/HG vs DIA. Data presented as mean±SEM (n=7). Data were analyzed by one-way ANOVA (a) and two-way ANOVA (b) with Bonferroni post-test. HG: hyperglycemia, DIA: diazepam, MDZ: midazolam.

HG/MDZ, HG/DIA and HG groups ($P>0.05$, Fig 5a). MSE analysis of IBI time series has shown that sample entropy of the IBI was significantly lower in MDZ and DIA groups compared to the control group (for scale 1–4 and scale 2-4, respectively; $P<0.05$). There was no significant difference in MSE of IBI among HG/MDZ, HG/DIA and HG ($P>0.05$). Moreover, MSE of IBI revealed higher entropies for scales 1-5 in HG/MDZ in compression with MDZ ($P<0.05$) and for scales 2,4,5 and 9-10 in HG/DIA ($P<0.05$) in comparison with DIA (Fig 5b).

Discussion

The aim of the current study was to investigate the effects of chronic HG on respiratory responsiveness to BZRAs. The major finding of this study is injecting intact animals with benzodiazepines changed their breathing pattern. That was associated with an

increase in respiratory rate, a decrease in entropy and an increase in the coefficient of variation of IBI, whereas, chronic HG decreased the respiratory effect of benzodiazepine receptor agonists. It has previously been reported that subjects with HG exhibit abnormal respiratory responses to a different range of stimuli (Williams et al., 1984; Weisbrod et al., 2005). This is the first study to report different breathing pattern responses to BZRAs in hyperglycemic rats.

Diabetic patients are susceptible to sleep related respiratory disorder and sleeping problems and anxiety. Management of sleeping problems and anxiety is important because it improves the quality of life. BZRAs are frequently used to treat subjects with insomnia and mental health problems, despite the concern of respiratory depression. Previous studies have demonstrated that intermittent hypoxemia (Reutrakul and Mokhlesi, 2017) and an altered pattern of breathing during sleep (Weisbrod et al., 2005) are more marked in patients with diabetes. In addition, BZRAs-induced respiratory suppression could persist for several hours, which may worsen sleep related respiratory disorders such as apnea (Sharafkhaneh and Hirshkowitz, 2012, Heck and Zolezzi, 2015) comorbidity with diabetes.

We evaluated respiratory pattern dynamics in response to BZRAs in intact and hyperglycemic animals using complexity analysis of respiratory rate. Change in the respiratory pattern following BZRAs administration in intact animals was associated with increased respiratory rate and IBI variability and decreased IBI entropy. Greater regularity (lower entropy) corresponds to increased isolation of respiratory components from the surrounding environment (Pincus and Goldberger, 1994). GABA receptor agonists could increase inhibition in the respiratory control center and this might change the adaptability of the respiratory system to a feedback mechanism. This finding is consistent with studies that have shown that the administration of BZRAs changes breathing pattern in healthy humans and animals (Forster et al., 1980; Faroqui et al., 1983; Morel et al., 1984; Berggren et al., 1987). Berggren et al. (1987) demonstrated that intravenous injection of MDZ and DIA in healthy subjects led to alteration in breathing pattern with an increase in respiratory rate and decrease in tidal volume. In addition, in the intact animal, alteration in respiratory parameters following midazolam injection was more than diazepam. This higher effect of midazolam on respiratory function may be related to the higher potency and efficacy of midazolam compared to diazepam (Cole et al., 1983). Also, the finding that the magnitude of increase in mean respiration rate and IBI variability, and decrease in entropy and mean of IBI was significantly less in HG/MDZ and HG/DIA compared to MDZ and DIA groups, suggests that hyperglycemic animals have an impaired response to BZRAs (Ramanathan et al., 1998). Respiratory parameters such as rate and volume (Benchetrit, 2000), continuously fluctuates around the physiological set point (Jain, 2011) to maintain adaptability to external or internal stimuli in healthy respiratory system (Thamrin and Frey, 2009). However, fluctuation in respiratory pattern in response to stimuli can be too rigid or loss of control whit disease state (Frey et al., 2011). Lower alteration in respiratory pattern entropy suggests that diabetes might be associated with greater distance from normal fluctuation and respiratory system become too stable and limited adaptability to environment alteration (Frey et al., 2011). Previous studies indicated that diabetic patients exhibit abnormal cardiopulmonary reflex responses to different stimuli (Smith, 1982; Nishimura et al., 1989). Weisbrod et al. (2005) have shown that diabetic subjects exhibit abnormal response to acute isocapnic hypoxia.

Besides, previous studies reported that alteration in drug effects in diabetic patients. Ramanathan et al. (1998) previously reported that the anxiolytic effect of diazepam was reduced in diabetic rats and anxiolytic activity was observed only at a higher dose. However, the mechanism underlying the blunted response to BZRAs in STZ-induced hyperglycemia rats is not clear. Some of the studies have shown that diabetic neuropathy is the probable mechanism for the loss of afferent integrity and respiratory disorder in these patients (Sobotka et al., 1986; Nishimura et al., 1989). Evidence from previous studies suggest that GABA plays a role in the pathophysiology of the diabetic neuropathy (Yasin Wayhs et al., 2015). Abnormal response to BZRAs might be the result of alteration in GABA receptor binding, as previously reported by Antony et al. (2010), who have shown that GABAA receptor α-subunits and GABAB receptor gene expression significantly decreased in diabetic rats. Also, this abnormality in the GABAergic

neurotransmitter system can be the result of increased GABA levels due to HG (Van Bussel et al., 2016). According to the evidence, chronic exposure of receptors to the ligands leads to increased down-regulation of receptors (Miller et al., 1988). Thus, increased GABA levels may have a similar effect on GABA receptors in diabetes patients, resulting in impaired response to BZRAs.

Since BZRAs can cause alteration in respiratory volume, evaluation of changes in respiratory volume would improve the current findings and interpretations. Also, as the previous studies have reported abnormal respiratory responses to hypoxia in diabetic patients (Weisbrod et al., 2005), blood gas measurement during respiratory signal recording might increase our interpretation of the current results.

Conclusion

The present study demonstrated an alteration in breathing pattern follow the BZRAs administration in the intact animal. Moreover, STZ-induced HG modulated adverse respiratory outcomes of BZRAs. According to this finding, it seems that chronic HG might induce alteration in benzodiazepine receptors response to the BZRAs.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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