Original Article

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The Effect of Streptozotocin-Induced Chronic Hyperglycemia on Respiratory Effects of Morphine in Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Neuropathic pain is a common complication of diabetes. Today opioid analgesics such as morphine, use for the management of neuropathic pain. Since the respiratory effects of morphine have not been studied in diabetes, the aim of this study was to investigate the effect of chronic hyperglycemia on the effects of morphine on respiration.

METHODS: 32 Male Wister rats were randomly allocated into control group (injection of citrate buffer as streptozotocin solvent), morphine group (injection of citrate buffer as STZ solvent and injection of morphine before respiratory recording) group, hyperglycemia group (STZ injection), and hyperglycemia-morphine group (STZ injection and morphine injection before respiratory recording). Hyperglycemia was induced by injecting streptozotocin (35 mg/kg, i.p.). Respiration was recorded by plethysmography after which respiratory volume, inter-breath interval, and respiratory rate were quantified using MATLAB.

FINDINGS: Mean of respiratory rate (in the hyperglycemia was 83±3.9 and control was 101±4 ($p<0.05$)), coefficient of variation of respiratory rates (in the hyperglycemia was 0.615±0.1 and control was 1.05±0.07 ($p<0.05$)), mean of respiratory volume (in the hyperglycemia was 1.44±0.19 and was control 1.1±0.11 ($p<0.05$)), and mean of inter-breath interval (in the hyperglycemia was 1.28±0.01 and control was 0.93±0.03 ($p<0.05$)). coefficient of variation of respiratory volumes (in the hyperglycemia-morphine was 1.14±0.16 and was morphine was 1.68±0.1 ($p<0.05$)), mean of respiratory rate (in the hyperglycemia-morphine was 1.24±0.34 and morphine was 0.37±0.04 ($p<0.05$)), and mean of inter-breath interval (in the hyperglycemia-morphine was 1.09±0.07 and morphine was 0.74±0.05 ($p<0.01$)).

CONCLUSION: Our results show that chronic hyperglycemic changed breathing pattern and respiratory response to morphine.

KEY WORDS: *Diabetes, Breathing Pattern, Morphine, Respiratory System.*

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Introduction

Breathing is a dynamic process in volume and number, created by the respiratory rhythm production center in the brainstem. In a healthy state, the dynamics of respiration fluctuate under the influence of stimuli from different inputs to maintain homeostasis and adapt to different types of stimuli in a physiological range (1, 2). This oscillating behavior in the respiratory pattern in diseases is out of the normal range, and in response to stimuli, the respiratory control system can become very difficult and unresponsive or out of control (3).

Diabetes mellitus is a common disease, affecting more than 415 million people worldwide in 2015, and is predicted to reach more than 642 million in 2040 (4). The vascular and neurological complications of diabetes have made diabetes one of the leading causes of death in the world. Studies have also reported high prevalence of neuropathic pain (5) and respiratory disorders such as sleep apnea in diabetic patients (6, 7). These disorders, along with the disease, reduce a person's daily functioning and quality of life (8).

Today, opioid analgesics such as hair receptor agonists are prescribed to relieve neuropathic pain in patients (9). Hair receptors are G-protein-coupled receptors that are expressed in the neuronal membrane of many parts of the nervous system (10). Hair receptor agonists such as morphine bind to the hair receptor, opening up potassium channels and hyperplasia and reducing neuronal irritability (11, 12).

Due to the widespread expression of morphine receptors in different parts of the brain, such as the brainstem, which is the center of respiratory rhythm production, as well as higher areas of the brain such as the thalamus, anterior cingulate cortex, and insula, which play a role in regulating respiration (13, 14), has led to the most important concern associated with morphine administration, its respiratory attenuation effect (15), which can exacerbate respiratory problems in patients and limit its use in clinic. Because diabetic neuropathy is one of the most common complications of diabetes and occurs in different parts of the nervous system (16), it leads to disruption of various systems of the nervous system and causes psychological diseases (17, 18), as well as disorders in responses to opioid receptors (19-24). According to the hair receptor expression in the respiratory control center, there is also a possibility of dysfunction of the hair receptor in these areas of the brain. Because morphine is needed to relieve neuropathic pain in diabetics, the effects of high blood sugar on diabetes on respiratory response following morphine administration have not been studied, so the aim of this study was to investigate the effect of chronic hyperglycemia induced by streptozotocin on respiratory response following injection of morphine in rats.

Methods

In this experimental study, after the approval of the Ethics Committee of Arak University of Medical Sciences with the ethical code of IR.ARAKMU.REC.1398.164, 32 male Wistar rats, in the weight range of 180-200 g were used. The animals were kept in a stable temperature, 12 hours of darkness and 12 hours of light and without restrictions on access to water and food, according to the ethical protocol of working with laboratory animals approved by Arak University of Medical Sciences. The animals were randomly divided into four experimental groups of 8. Control group (citrate buffer injection as a solution of streptozosin (STZ)), morphine group (citrate buffer injection as STZ, morphine injection before respiratory recording), hyperglycemic group (STZ injection) and hyperglycemic-morphine group (STZ injection, morphine injection before respiratory recording).

Hyperglycemia induction: Streptomycin (STZ) purchased from Sigma USA was used to create hyperglycemia. The required STZ dose for hyperglycemia was selected based on a previous study (25) and its effectiveness was confirmed during a pilot study. In order to induce hyperglycemia, 35 mg/kg of STZ dissolved in 0.1 mol/l citrate buffer was injected intraperitoneally. Four days after STZ injection, the induction of hyperglycemia in animals was confirmed by measuring blood glucose with a glucometer. Rats with a blood glucose concentration higher than 200 mg/dl were considered hyperglycemic. Animals with glucose concentrations above 350 mg/dl were also excluded.

Respiratory recording: In this study, respiratory recording was performed in two stages. The first step was to record the base, which was done at the beginning of the experiment and before the STZ or citrate buffer injection. The second stage was at the end of the experimental period, which was in the hyperglycemic

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and hyperglycemic-morphine groups three weeks after the hyperglycemia was established and in the control and morphine groups three weeks after baseline recording. In the morphine and hyperglycemia-morphine groups, 10 mg/kg morphine (anti-pain dose) (26) was injected intraperitoneally before the second phase of respiration was recorded and 15 minutes later the respiratory recording was performed. Respiration recording was performed by polysystemography (BIODAC-R172, Trita Wavegram Co., Iran). The recording device consisted of a Plexiglas cylinder (length 30 cm, diameter 7.5 cm and volume L 1.32). Air was pumped into the cylinder through a 4 L/min input on one side and the cylinder was connected to a pressure converter in the recording device through an outlet using polyethylene pipe. The data was displayed in proprietary software and stored on a computer (27).

In order to allow the animals to get used to the recording cylinder and reduce stress, the animals in all experimental groups were placed in the breathing recording cylinder for 30 minutes daily for three consecutive days. The recording time for each animal was 30 minutes, which included 10 minutes for the animal to become accustomed to the conditions in the cylinder and 20 minutes for recording the breath for analysis.

Respiratory pattern analysis: Twenty minutes of the recorded respiratory signal were used to perform the respiratory pattern analysis. The method of calculating the intervals between breathing, respiratory volume and number of breaths for analysis in MATLAB software was such that first the peak of respiratory waves is identified and marked. Then, to evaluate the changes in respiration pattern, parameters such as fractal correlation, irregularity, coefficient of change and average volume and intervals between breaths were evaluated. Detrended fluctuation analysis (DFA) method was used to investigate fractal correlation, which shows the fractal correlation of a series of time at different time scales. In this method, the rate of change in different time scales is calculated and shown linearly on the logarithmic graph which horizontal axis is the logarithm of scale and vertical axis is the logarithm of the variance changes. The slope of this line is an estimate of the fractal correlation (28), so that if the slope of the line is equal to one, it shows the maximum fractal correlation. Sample entropy (sampEn) and Cross SampEn methods were used to calculate the amount of irregularity in time series which is less calculated values indicate a decrease in irregularity in the breathing pattern (29).

Statistical tests: Data analysis was analyzed using Graph pad prism 6 statistical software and was displayed as the mean±error. One-way analysis of variance was used to evaluate the data on changes in respiratory volume, the interval between respiration and the number of breaths between groups, and the Toki test was used to determine the groups with differences. Unpaired t-tests were used to evaluate blood glucose concentration data and $p<0.05$ was considered significant.

Results

Examination of changes in respiratory volume between groups showed that there was a significant difference between the mean values of respiratory volume in hyperglycemic group (1.44±0.19) and in control group (1.1±0.11) ($p<0.05$) and DFA of respiratory volume in hyperglycemic group (1.16±0.03) and in the control group (0.98±0.05) ($p<0.05$). The results also showed that between the mean respiratory volume of hyperglycemic-morphine group (1.24±0.34) and morphine group (0.37±0.04) ($p<0.05$) and the coefficient of variation in respiratory volume of hyperglycemic-morphine group (1.14±0.16) and the morphine group (1.68±0.1) ($p<0.05$) were significantly different (Figure 1).

The study of the differences between breathing intervals showed that there is a significant difference between the mean respiratory intervals in the hyperglycemic group (1.28±0.01) and the control group (0.93±0.03) ($p<0.05$) and DFA of respiratory intervals in the hyperglycemic group (1.14±0.02) and in the control group (0.91±0.02) ($p<0.01$). The analysis of the results also showed a significant difference between the mean respiratory intervals in hyperglycemic-morphine group (1.09±0.34) and in morphine group (0.74±0.04) ($p<0.05$). In examining the changes in the number of breaths, there was a significant difference between the average respiration rate of the hyperglycemic group (83±3.9) and in the control group (101±4.) ($p<0.05$) and the coefficient of variation in the respiration rate in the hyperglycemic group (0.615±0.1) and the control group (1.05±0.07) ($p<0.05$) (Figure 2).

The results of comparing the glucose plasma concentration showed a significant difference between the hyperglycemic group and the control (293±13 mg/dl, 132±6 mg/dl $p<0.001$, respectively).

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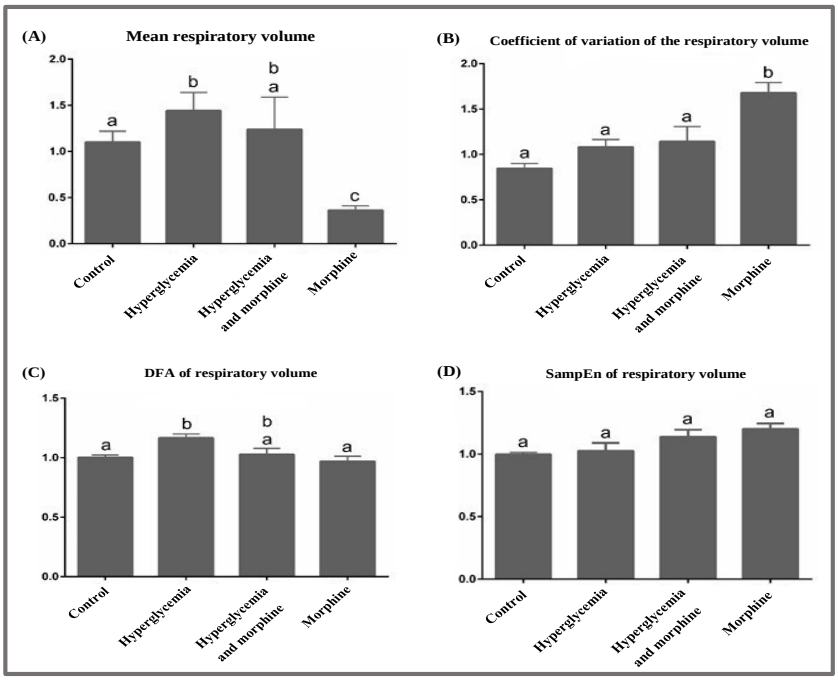


Figure 1. Comparison of changes in respiratory volume in experimental groups

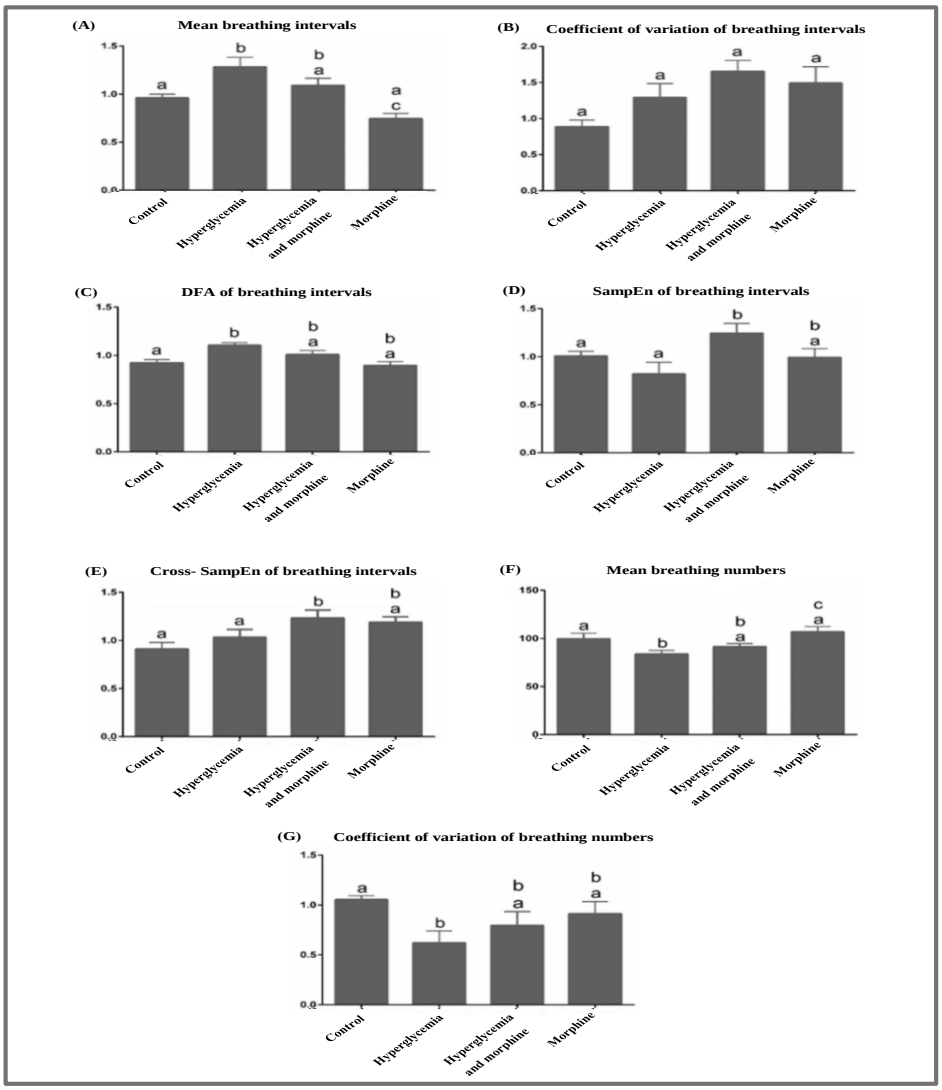


Figure 2. Comparison of changes in breathing intervals and number of breaths in experimental groups

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Discussion

The results of the study showed that chronic hyperglycemia induced by STZ alters the pattern of respiration; this change in respiratory pattern was associated with a decrease in the mean and coefficient of variation in the number of breaths, as well as an increase in the mean and decrease in fractal correlation between volume and breathing intervals. In addition, the results showed that in hyperglycemic animals, the respiratory effects of morphine decreased, which was characterized by a lower decrease in mean respiratory volume and intervals between breaths and a decrease in the coefficient of variation of respiratory volume.

The results of the study showed that chronic hypertension causes a change in the respiratory pattern of animals in the waking state. Previous studies have reported that ventilatory responses to different stimuli are different in diabetic people (6, 30). Also, the breathing pattern during sleep of diabetic people is different from normal people (7). The results of respiratory assessments in diabetic patients under normal conditions have shown that there is no significant difference between the volume of minute ventilation between diabetic and non-diabetic patients (6), but in response to stimuli such as hypoxia, hypercapnia and exercise, diabetic people show different breathing responses compared to normal people (6, 30). Changes in breathing pattern during sleep in diabetics can be due to insulin resistance or chronic hyperglycemia (31).

The results of Malone et al.'s study showed that chronic hyperglycemia increases the concentration of compounds within neurons that change the neuronal structure and dendrites of neurons by changing the osmotic pressure (32). Hyperglycemia can also alter the expression of receptors (33) and induce oxidative stress in cells (34), which can lead to changes in the activity of cells and neurons. Similar events may occur in neurons and cells associated with the respiratory control center as a result of hyperglycemia, which causes the control center neurons to respond abnormally to various stimuli and to develop abnormal breathing patterns in these patients (6, 30).

Our results showed that morphine injection in healthy animals altered the pattern of respiration in animals. This is consistent with the results of previous studies, which have shown that morphine causes slow and irregular breathing (35, 36). Our results also showed that changes in respiratory dynamics in response to morphine injection were less common in hyperglycemic animals than in healthy animals. The results of previous studies have shown that Diabetes reduces the analgesic effects of morphine in humans and animal models of diabetes (19, 20, 23, 24).

A study by Simon et al. showed that the analgesic effects of morphine were reduced in diabetic rats compared to control animals. They reported that the reason for this change in morphine response was not due to differences in the uptake, distribution, concentration of morphine in the brain, or removal of morphine in diabetic animals (19). Hajializadeh et al. also showed that the response to morphine in diabetic animals decreased when morphine was injected into the brain ventricles of these animals. Decreased morphine receptor sensitivity and changes in the expression pattern of cellular components such as receptors have been suggested as possible causes of changes in morphine response in diabetic animals (33).

According to the results of the studies mentioned above, hyperglycemia may changes the response to morphine by affecting neuronal cellular components (33). Since the respiratory control system is also part of the nervous system, it is possible that hyperglycemia in this part of the nervous system has similar effects on the opioid system, which causes changes in respiratory dynamics and respiratory response to morphine.

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