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Action potentials of the superior cervical ganglion neurons in the rats in diabetes mellitus

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It's well known that sympathetic and sensory neurons are affected in the early stages of diabetes *mellitus (DM)*. However, the functional disorders that occur in neurons of the superior cervical ganglion (SCG) under the conditions of DM remain insufficiently studied. Therefore, the aim of this study was to *evaluate* the effect of streptozotocin-induced diabetes mellitus (DM) of the rats on action potentials (AP) recorded in the superior cervical ganglion`s (SCG) neurons. Rats with blood sugar level more than 30 mM were taken into experiment. The SCG of healthy control rats (n=12), rats at week 4 (n=9), and rats at week 12 after streptozotocin injection (n=9) were studied in vitro. AP of the SCG neurons were registered by the microelectrode technique. Neurons of the SCG were stimulated directly with 150 ms depolarizing current in pulse of 100 pA. The AP parameters of 36 SCG neurons of control rats were alternately compared with the corresponding AP parameters of 22 neurons of rats at week 4 and 30 SCG neurons of rats at week 12 after streptozotocin injection. The data obtained demonstrate that the AP amplitude and overshoot of AP, maximum rise and fall rates, and afterhyperpolarization amplitude significantly decreased at 12 weeks after DM induction. At the same time, the rheobase value significantly increased, this may indicates decreasing of the neurons plasma membrane excitability. Only the AP maximum rate of fall decreased statistically significant at week 4, the maximum rate of rise had an insignificant tendency to decrease at that time. However, the resting membrane potential and excitation threshold didn't *change even* at 12 weeks after the injection. Thus, functional disorders of rat SCG neurons were appeared at a quite late stage of DM. The differences in AP parameters may result from neurons' membrane ionic conductivity *alterations*, decreasing of its excitability and reducing ion channels efficiency in later stages of DM. This suggests that SCG is an important target of pathophysiological disorders caused by DM.
Key words: superior cervical ganglion; diabetic neuropathy; streptozotocin-induced diabetes; action potential; membrane conductivity.

INTRODUCTION

Although recent advances in the treatment of diabetes mellitus (DM) improve the health state of patients, the long-term effects of DM still lead to further complications. One of them is chronic diabetic autonomic neuropathy [1]. The mechanism of the neuropathy development is the damaging of small blood vessels of the sympathetic and parasympathetic divisions of the autonomic nervous system. Neurons of the ganglia are affected by ischemia, oxidative stress. It leads to damaging of the nerve fibers, disruption the formation of nerve growth factor. The polyol pathway activation and plasma membranes glycoly-

sis are observed [2]. However, the underlying causes of diabetic autonomic neuropathy remain poorly understood. Its pathological mechanisms can be studied using experimental animal models. DM in animals is caused by specific drugs such as alloxan and streptozotocin, which have a cytotoxic effect on the pancreas β-cells leading to chronic diabetic hyperglycemia [3]. One of the typical objects for the autonomic nervous system disorders study is the superior cervical ganglion (SCG). As the upper extremity of the sympathetic trunk, it plays an important role in the body, SCG provides cervical, facial, and intracranial structures innervation. It innervates pineal and pituitary glands, carotid body, thyroid

and parathyroid glands, iris, eyelids, etc. It has postganglionic fibers that are responsible for innervating the heart [4]. It is known that DM causes neuronal disorders in SCG, affecting the excitability of neurons and synaptic transmission to them [5]. In addition, the intensity of the blood supply to the SCG reduces [6]. Also, it was found the activity of Na^+- K^+-ATPase of the plasma membrane of the SCG neurons significantly decreased [7]. Previous studies in rats with DM have shown significant decrease for AP amplitude and spike after-hyperpolarization amplitude of the SCG neurons. Moreover, resting membrane potential and input resistance of the SCG neurons significantly decreased too. However, other parameters, such as rheobase, AP threshold, and duration, remained unchanged in rats with DM [7]. This underscores the complexity of DM-induced alterations in neuronal function and highlights the need for further investigation to fully understand these mechanisms. The general mechanism of diabetic disorders remains unclear.

The aim of our study was to determine the features of action potentials (AP) that arise in response to direct stimulation of SCG neurons in rats with experimental streptozotocin-induced type 1 DM at the different stages of its duration.

METHODS

Male Wistar rats 3 to 4 months old with a blood glucose level less than 8 mM in control were selected for the experiment. DM was simulated by a single intraperitoneal injection of streptozotocin at a concentration of 65 mg/kg. It results to persistent diabetic hyperglycemia in rats. Animals were kept in conditions of free access to food and water. Their blood sugar levels were measured with an On-Call Plus glucometer (USA). Rats with DM with blood sugar level more than 30 mmol/l were taken into experiment in the 4th and 12th weeks after injection, divided into appropriate groups. All experiments were conducted in compliance with ethical norms and requirements of European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

The SCG of the rats was removed, cleared of the connective tissue membrane and fixed to the bottom of the electrophysiological chamber with the tungsten wire a 30 μm in diameter. The experiments were performed in vitro in physiological solution of the following composition (mmol/l): NaCl - 140; KCl - 2.2; $CaCl_2$ - 2.6; $MgCl_2$ - 2; HEPES - 10; glucose 30; pH 7.4 (pH was adjusted by adding NaOH).

The potential of SCG neurons was recorded by the microelectrode technique using the amplifier Axoclamp 2B Current and Voltage Clamp ("Axon Instruments", USA) in the "bridge" mode. Differential interference contrast microscope ("Carl Zeiss Jena", Germany) with a water immersion lens with a 400× total magnification was used for visual inspection. The signals were digitized by a Digidata 1200A analog-to-digital converter controlled by the Clampfit 9.0 computer program ("Axon Instruments", USA). Microelectrodes were made of glass tube with a 1 mm in diameter of borosilicate refractory glass company "Pyrex" using a Flaming-Brown puller P-97 (Flaming/Brown Micropipette Puller, "Sutter Instrument Co.", USA). The resistance of the microelectrodes was 65 ± 20 MOhм. They were filled with KCl solution at a 1.5 mmol/l concentration. AP was caused by direct stimulation of SCG neurons with rectangular depolarizing current pulses of 150 ms in duration with increasing amplitude by 100 pA by step. The interval between stimuli was 5 s. The results were processed from neurons with resting membrane potential exceeded -40 mV.

AP from 36 SCG neurons of the control rats (n = 36), 22 neurons at week 4 (n = 22), and 30 neurons at week 12 after streptozotocin injection of the rats with DM (n = 30) were analyzed. AP parameters such as amplitude, excitation threshold, overshoot, maximum rate of rise and maximum rate of fall were calculated. Rheobase was defined as the value of the threshold current which occurs AP. The amplitude of afterhyper-

polarization relative to the excitation threshold was also measured. Statistical analysis was performed using Origin 8.5 ("Microcall Inc.," USA). The obtained results were compared using Student's t test. The averages were considered to differ significantly from each other when the p value for the occurrence of the null hypothesis was P ≤ 0.05. The results are presented as mean ± standard error of mean.

RESULTS

The values of the resting membrane potential of SCG neurons in the control group of rats and in rats with experimental streptozotocin-induced DM were analyzed. It was -48.8 ± 1.1 mV in the control, -49.9 ± 1.6 mV at week 4 and -49.1 ± 1.3 mV at week 12 after streptozotocin injection. No statistical differences were found between these values. The average rheobase in control rats was 180 ± 20 pA, in rats at the 4th week of DM 190 ± 21 pA, and at the 12th week 280 ± 25 pA. A statistically increase in rheobase relative to control was observed at week 12 after streptozotocin injection, which may indicate a reduction in neuronal excitability in the late stage of DM. The AP parameters of SCG neurons caused in response to direct stimulation of the neuron soma by rectangular depolarizing current pulses were calculated (Fig. 1A). It was found that the mean value of the amplitude of AP in rats at 12 weeks after streptozotocin injection was significantly reduced by 5.4 mV relative to control, but at 4 weeks this figure did not differ statistically from control (Fig. 1B). The amplitudes of the AP decreased due to decreasing of the overshot. The excitation threshold did not change significantly in DM and was -23.8 ± 0.9 mV in the control, -23.4 ± 1.0 mV and -24.2 ± 0.9 mV at week 4 and 12 accordingly. The amplitude of afterhyperpolarization also decreased significantly only at 12 weeks after streptozotocin injection. Its mean value reduced by 2.1 mV relative to control (Fig. 1C).

The maximum rate of rise and the maximum rate of fall of AP was calculated by response differentiation (dV/dt). The AP differential consisted of positive and negative peaks, the value of which is proportional to the maximum amplitudes of the depolarizing Na^+- and repolarizing K^+-currents according to the I = C (dV/dt) formula, where C is the membrane capacity. It was found that the maximum rate of rise of AP in rats with DM decreased significantly relatively to the values in healthy rats, by 13.8 mV/ms in the 4th and by 21.5
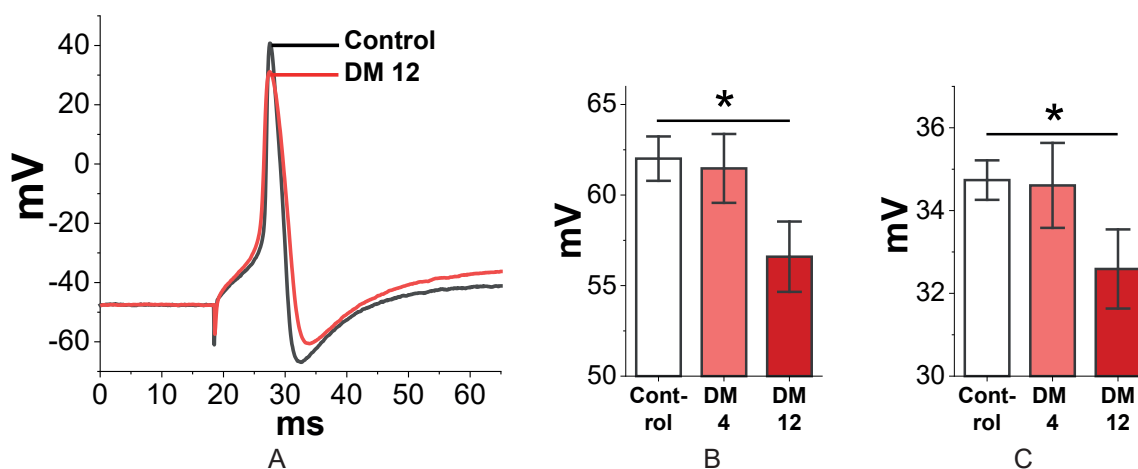

Fig. 1. Action potentials of the superior cervical ganglion neurons in healthy rats (control), rats with diabetes mellitus at week 4 (DM 4) and at week 12 (DM 12) after streptozotocin injection. A - Action potentials caused in response to direct stimulation of the neuron soma by rectangular depolarizing current pulses. B - amplitudes of action potentials. C - amplitudes of afterhyperpolarization measured relative to the excitation threshold *P ≤ 0.05

mV/ms in the 12th week after DM induction. However, the differences were statistically only at week 12 (Fig. 2A). The maximum rate of fall in rats with DM also decreased relatively to the control by 3.8 mV/ms and 3.6 mV/ms in the respective groups. Significant differences were found even at week 4 after streptozotocin injection (Fig. 2B).

DISCUSSION

Our findings indicate that the action potential (AP) parameters in SCG neurons of rats undergo significant changes in our diabetic model. These alterations may stem from complex disruptions in plasma membrane ionic conductivity, decreased neuronal excitability, or diminished ion channel efficiency, likely due to prolonged metabolic disturbances. They suggest that SCG is an important target of pathophysiological disorders caused by DM. The increasing of the SCG neurons rheobase, decreasing of the AP amplitude and their maximum rate of rise at 12 weeks after streptozotocin injection may indicate dysfunction of potential-dependent Na^+ channels or reduction of the membrane gradient of Na^+ [8]. At the same time, decreasing of the afterhyperpolarization amplitude at week 12 of

DM and the AP maximum rate of fall at week 4 may indicate an alteration of the potential-dependent K^+ channels. It is known that blocking K_v2 and K_v4-potassium channels leads to an increase in the duration of repolarization and AP maximum rate of fall. As a result, more calcium ions enter to the neuron, which might cause cytotoxic effects [9]. Decreasing of the afterhyperpolarization amplitude may also be due to the influx of Na^+ or Ca^{2+} into the neuron, or the outflow of Cl^- during repolarization of its plasma membrane. Decreasing the activity of the Na^+-K^+-ATPase, at least in relation to the sodium ions transfer from the intracellular environment after depolarization, also leads to inhibition of afterhyperpolarization [10]. In addition, there are studies that indicate dysfunction of Na^+-K^+-ATPase of SCG neurons of mice and rats in DM [5, 7]. However, such alteration would lead to reduction of the K^+ concentration gradient and to decreasing of the neurons resting membrane potential [11]. But in our study, the SCG neurons resting membrane potential of rats in DM did not differ from control values.

Previous biochemical studies have shown that aldose reductase inhibitors such as sorbinil prevents decreasing of Na^+-K^+-ATPase activity in rats in DM. Under the DM conditions, it synthesizes in excess and causes osmotic damage of SCG neurons [12]. It is also supposed that the increase in glucose concentration of neurons in DM causes an increase in the NADH and FADH2 production due to glycolysis and citric acid cycle alterations. Due to the increasing of electron donors' concentration, the electron transport chain is blocked in complex III [13]. Thus, the increase in the concentration of NADH and FADN2 leads to an increase in the number of reactive oxygen species, shifting the intracellular thiol/disulfide redox equilibrium towards oxidative conditions, causing oxidative stress in neurons [14]. It could be assumed that these alterations may be because of free oxygen radicals. Thus, in experiments on mice, it was shown that DM caused oxidation of the nicotinic acetylcholine receptors α3 subunit, leading to changes in the
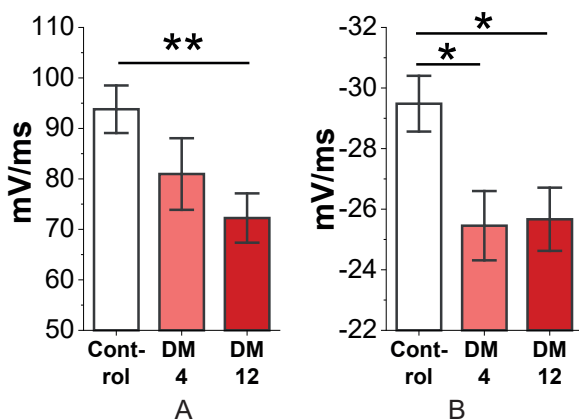

Fig. 2. Properties of action potentials of the superior cervical ganglion neurons in healthy rats (control), and rats with diabetes mellitus at week 4 (DM 4) and at week 12 (DM 12) after streptozotocin injection. A - maximum rate of rise, B - maximum rate of fall. *P ≤ 0.05; **P ≤ 0.01

amplitude characteristics of acetylcholine receptor current [5]. It is possible that oxidative stress under the conditions of DM in a similar way affects the potential-dependent ion channels or Na^+-K^+-ATPase in rats.

CONCLUSIONS

At steadily elevated blood glucose levels in rats with DM at 12 weeks after streptozotocin injection, the amplitude, overshoot, and maximum rate of rise of AP of SCG neurons decreased significantly, and the rheobase increased. However, such parameters did not differ from the control values at week 4 after injection. The amplitude of afterhyperpolarization of AP also significantly decreased at 12 weeks and the AP maximum rate of fall decreased at 4 and 12 weeks after streptozotocin injection. Passive electrophysiological properties of SCG neurons as membrane resting potential and their excitation threshold did not differ significantly from the control values even at week 12 after streptozotocin injection. Our current findings show that there may be additional or previously underappreciated changes in neuronal excitability in the diabetic state. This highlights the complexity of DM-induced alterations in neuronal function and underscores the need for further investigation to fully understand these mechanisms. They suggest that SCG is an important target of pathophysiological disorders caused by DM.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ПОТЕНЦІАЛ ДІЇ НЕЙРОНІВ ВЕРХНЬОГО ШИЙНОГО ГАНГЛІЯ У ЩУРІВ ПРИ ЦУКРОВОМУ ДІАБЕТІ

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Відомо, що симпатичні та сенсорні нейрони вражаються на ранніх стадіях цукрового діабету (ЦД). Однак функціональні порушення, що виникають у нейронах верхнього шийного ганглія (ВШГ) за умов ЦД залишаються недостатньо вивченими. Тому метою роботи було дослідити вплив стрептозотоциніндукованого цукрового діабету (ЦД) на потенціали дії (ПД) мембрани нейронів верхнього шийного ганглія (ВШГ) у щурів. До експерименту були відібрані щури, вміст глюкози у крові котрих перевищував 30 ммоль/л. Щурів із ЦД відбирали на 4-му тижні (1-ша група, n = 9) та щурів на 12-му тижні (2-га група, n = 9) після ін'єкції стрептозотоцину. Здорові щури (n = 12) ввійшли до контрольної групи. ПД нейронів ВШГ реєстрували мікроелектродним методом. Нейрони ВШГ стимулювали безпосередньо деполяризувальни імпульсами струму тривалістю 150 мс, збільшуючи амплітуду з кроком 100 пА. Інтервал між стимулами становив 5 с. Параметри ПД 36 нейронів ВШГ контрольних щурів порівнювали з відповідними параметрами ПД 22 нейронів щурів 1-ї дослідної групи нейронів ВШГ щурів 2-ї групи. Виявлено, що амплітуда, овершут ПД, його час наростання та спаду і амплітуда слідової гіперполяризації достовірно зменшувалися на 12-й тиждень після індукції ЦД. При цьому реобаза достовірно зростала, що вказує на зниження збудливості мембрани нейронів ВШГ. На 4-й тиждень лише швидкість спаду ПД вірогідно знижувалася, а швидкість наростання виражала тенденцію до зниження. Однак мембранний потенціал спокою і поріг збудження суттєво не змінювалися навіть на 12-й тиждень після ін'єкції стрептозотоцину. Отже, функціональні порушення нейронів ВШГ щурів проявлялися на достатньо пізніх етапах ЦД. Наведені відмінності показників ПД можуть бути зумовлені комплексними порушеннями іонної провідності мембрани нейронів, погіршенням їх збудливості, зменшенням ефективності іонних каналів на пізніх стадіях ЦД. Це свідчить про те, що ВШГ є важливою мішенню патофізіологічних порушень, спричинених впливом ЦД.

Ключові слова: верхній шийний ганглій; діабетична автономна нейропатія; стрептозотоциніндукований цукровий діабет; потенціал дії; провідність мембрани.

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