Diabetic peripheral neuropathy (DPN) is the most severe and widely distributed complication of diabetes mellitus (DM). DPN may be diagnosed in more than half of patients with long history of the disease [1]. The most common manifestation of DPN is symmetrical distal sensorimotor polyneuropathy, which is observed in 70% of cases [2]. Specific early signs of DPN are impairments of various types of sensitivity including nociception, which appear in distal parts of extremities and expand to their proximal parts. DPN is a chronic and progressive disorder. Modern therapy is aimed at prevention of progress of complications, but does not reduce developed degenerative changes [3]. In the case of insulin-dependent DM (type 1; DM1), the etiology of DPN has been well studied and considered to consist in metabolic impairments in nerves, which appear due to deficit of neurotrophic effects of insulin and C-peptide followed by long-term hyperglycemia [4]. Control of glucose level decreases the probability of DPN development by 60–70% [5]. However, studies on the early markers of development of diabetic neuropathy and its pathogenic mechanisms, specifically, neuropathic pain, are urgent problems of modern physiology and medicine.

Signal systems, such as the adenylyl cyclase signal system (ACSS), play an important role in regulation of pain sensitivity. The ACSS is involved in the regulatory effects of various hormones on muscle tissue and vessels supplying blood to it [6, 7]. To date, studies on the relationships between DPN and functional activity of the ACSS in muscle tissue are absent. The purpose of the present study was to examine the time course of changes in the threshold of peripheral nociception in male rats with 30-day streptozotocin-induced DM1 and to compare these changes with functional activity of the ACSS in skeletal muscles of diabetic rats. The model of streptozotocin-induced DM1 was chosen because it is associated with clear specific signs of DPN, including spontaneous pain, hyperalgesia, and allodynia [8].

Male 4.5-month-old Wistar rats were used for the study. DM1 was induced by a single injection of streptozotocin (Sigma, United States) dissolved in acidified 0.9% NaCl (pH 4.5) at a dose of 50 mg/kg. Control animals were injected with isotonic saline. Glucose level was measured in the blood sampled from the tail vein using One Touch Ultra test strips (United States) and a Life Scan glucometer (Johnson and Johnson, Denmark).

After a preliminary three-day testing, the animals were divided into two groups: the control group (C) consisting of 10 rats with a mean body weight of 341 ± 11 g and fasting glucose concentration of 5.6 ± 0.3 mM and the diabetic group (D) consisting of 8 rats with a mean body weight of 339 ± 7 g and fasting glucose concentration of 5.6 ± 0.3 mM. At the end of the experiment, the mean body weight and fasting glucose concentration were 361 ± 10 g and 5.6 ± 0.2 mM in group C and 347 ± 15 g and 17.5 ± 3.8 mM in group D, respectively.

Peripheral nociception was estimated using the Randall–Selitto test of mechanical pain tolerance [9]. Measurement of the sensitivity thresholds of mechanical stimulus was performed using an Analgesy-Meter (Ugo Basile, United States) with gradually increasing...
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pressure applied to dorsal surface of the rat hindlimbs. A rate of linear force elevation was 16 g/s and maximum force applied to a limb was 250 g. Measurement of a pain threshold was performed in five trials; in each trial, the test was performed in both hindlimbs at an interval of 20 s. The threshold of nociception was measured as a force applied to a hindlimb and expressed in grams at which the pulling-out response appeared. In each group, statistically significant differences between the thresholds estimated in the experiment and the differences between the groups were calculated using Student’s t test for dependent and independent variables, respectively. The differences were considered significant at p ≤ 0.05. Data are presented as M ± S.E.M.

The fraction of plasma membranes was separated from the hindlimb muscles (m. gastrocnemius), and the activity of adenylyl cyclase (AC, EC 4.6.1.1) was measured in it as described previously [10]. AC activity was estimated by measuring the content of cAMP formed in the enzymatic reaction and expressed as pmol of cAMP/(min mg of membrane protein). Statistical analysis was performed using ANOVA. Data of three independent experiments are presented as M ± S.E.M. The differences were considered significant at p ≤ 0.05.

During the three-day preliminary testing, we revealed that, in healthy rats, the basal nociception threshold was 112.4 ± 4.8 g. Two days after streptozotocin administration, development of hyperglycemia was accompanied by a clear decrease in the nociception threshold. Starting from day 10, the differences in the nociception thresholds in groups D and C were statistically significant (Fig. 1). Development of DM1 was accompanied by an additional decrease in the nociception threshold; 30 days from the start of the experiment, the nociception threshold in group D was about 79.3% of that observed in group C. These data show an early decrease in the peripheral nociception threshold under the conditions of experimental DM1 and indicate involvement of impairments of hormonal signaling in development of DPN. According to our data, these impairments appear at the early stage of DM1 and are associated with acute hyperglycemia and insulin deficiency [10, 11].

Studies on ACSS in the skeletal muscles of rats were performed at day 30 of DM1. Basal AC activity in plasma membranes of the skeletal muscles was 41.3 ± 3.6 pmol cAMP/(min mg of membrane protein), which was 35% higher compared to the muscles of control animals, where it was 30.7 ± 1.2 pmol cAMP/(min mg of membrane protein). Forskolin stimulates AC directly acting on the catalytic site of the enzyme and stimulating forskolin effects on AC were similar in groups D and C. We also induced AC activity with the nonhydrolyzed analogue of GTP guanylyl imidophosphate, which activates heterotrimeric Gs-proteins, stimulating AC, and the agonists of β-adrenergic receptors isoproterenol and BRL-37344. However, an increase in the AC activity in group D was substantially lower compared to group C. Stimulation of AC activity in the skeletal muscles of diabetic animals with the peptide hormone relaxin was about 41% of that observed in group C (Fig. 2). These data show a decrease in AC sensitivity to guanine nucleotides and hormones activating AC though catalytic functions of the enzyme remain preserved or even enhanced.

It is known that the adrenergic agonists acting via Gs-protein coupled β3-adrenoreceptors are involved in myogenesis and regeneration of skeletal muscles,