Intranasal insulin affects adenyl cyclase system in rat tissues in neonatal diabetes

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Abstract: The changes in hormone-regulated adenyl cyclase (AC) signaling system implicated in control of the nervous, cardiovascular and reproductive systems may contribute to complications of diabetes mellitus (DM). We investigated the functional state of AC system in the brain, myocardium, ovary and uterus of rats with neonatal DM and examined the influence of intranasally administered insulin on the sensitivity of this system to biogenic amines and polypeptide hormones. The regulatory effects of somatostatin and 5-HT, R-agonist 5-nonyloxytryptamine acting via G protein-coupled receptors were significantly decreased in DM and partially restored in insulin-treated rats. The effects of hormones, activators of AC, are changed in tissue- and receptor-specific manner, and intranasal insulin restored the effects rather close to the level in control. In insulin-treated non-diabetic rats, AC stimulating effects of isoproterenol and relaxin in the myocardium and of human chorionic gonadotropin in the ovaries were decreased, while the effects of hormones, inhibitors of AC, were increased. These data indicate that with intranasal insulin, G protein-mediated signaling pathways continue to gain strength. The obtained data on the influence of hormones on AC system in the brain, myocardium, ovary and uterus allow looking anew into the mechanisms of therapeutic effects of intranasal insulin.

Keywords: Adenylyl cyclase signaling system • Brain • Diabetes mellitus • Gonadotropin • Insulin • Myocardium • Ovary • Somatostatin • Uterus

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Abbreviations:

AC - adenyl cyclase;
AC system - adenyl cyclase signaling system;
DM - diabetes mellitus;
EMD-386088 - 5-chloro-2-methyl-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole;
GppNHp - b,g-imidoguanosine-5'-triphosphate;
5-HT receptor - 5-hydroxytryptamine receptor;
hCG - human chorionic gonadotropin;

1. Introduction

Hypertension, coronary heart diseases, atherosclerosis, nephropathy, retinopathy, neuropathy, cognitive deficit and disorders of the reproductive system are the most common complications of diabetes mellitus (DM) [1-3]. Chronic hyperglycemia is quite often an important contributing factor in the development of these complications. Many hormone- and growth factor-regulated signaling pathways, such as the adenyl cyclase (AC) signaling system, phospholipase C/protein kinase C signaling system and the cascade of mitogen-activated protein kinases are implicated in the regulation of the cardiovascular, nervous and reproductive systems and aberration of their functional activity in DM may contribute to complications of this disease [4-8]. It has been shown that in experimental types 1 and 2 DM the functional activity of hormone-sensitive AC system in the skeletal muscles, myocardium, testis, ovaries, uterus and brain of diabetic rats and the sensitivity of this system to hormones regulating AC activity are changed in tissue- and hormone-specific manner [6-12]. The most significant changes of hormonal sensitivity of AC system were found in the myocardium, testis and
The present work was undertaken with a view to study how intranasal delivery of insulin affects the hormone-sensitive AC system in the brain and peripheral tissues of female rats with neonatal model of type 2 DM similar by some characteristics to insulin-insensitive type 2 DM. We investigated the effects of 5-week treatment with intranasal insulin on the basal and hormone-regulated AC activity and G protein GppNHp-binding in the brain, myocardium and the tissues of reproductive system (uterus, ovaries) of diabetic animals, and compared them with those of diabetic rats without insulin therapy and with control animals. To estimate the tissue and hormone specificity of changes in DM in each case, we studied AC effects of hormones activating the enzyme via Gs protein-coupled receptors and hormones that, on the contrary, decrease AC activity and stimulate Gi proteins.

2. Experimental Procedures

2.1 Animals

For experiments, adult female Wistar rats housed in plastic sawdust-covered cages with a normal light–dark cycle and free access to food and water were obtained. The experiments were carried out under the guidelines of the National Institutes of Health regulations for the Care and Use of Animals for Scientific Purposes. All efforts were made to minimize animal suffering and reduce the number of animals used.

Neonatal insulin-independent DM was provoked by intraperitoneal administration of streptozotocin (STZ) dissolved in 0.9% NaCl solution, pH 4.5, at the dose of 80 mg/kg of body weight in newborn (5-day-old) rats [17]. Animals of control groups received acidified physiological solution. Using glucose tolerance test we showed that 5–6 month old rats with a neonatal model of DM have pronounced insulin resistance, typical of insulin-independent DM. It was shown that 2 hours after glucose loading (2 g/kg of body weight) in control rats, the concentration of sugar in blood reached a normal level, while in diabetic rats it did not. The glucose measurements in the whole blood from the tail vein were performed using test strips (One Touch Ultra, USA) and a glucometer (Life Scan Johnson & Johnson, Denmark). Diabetic rats had glucosuria. The monitoring of glucose in the urine was determined using test strips (Combi-Screen Analytica, Germany). Alongside, we observed a moderate hypoinsulinemia in rats with neonatal DM, which is due to damage of β-cells in the neonatal period of rat development [18]. The insulin concentrations in the serum of diabetic and control rats were 0.6±0.3 and 1.1±0.2 μg/L, respectively. The insulin concentration

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ovaries while in the brain the changes were not so pronounced, if affected at all. In most tissues of diabetic animals the effects of hormones acting on AC via G proteins of the inhibitory type (Gi) were changed to a greater degree compared with those of hormones acting on the enzyme via G proteins of the stimulating type (Gs), which is likely to be due to a decrease of protein expression and a reduction of their functional activity. Generally, in DM changes in the expression of some Gi- and Gs-coupled receptors, β-adrenergic receptors in particular, regulating the AC activity are also observed. However, the function of AC, catalytic component of AC system, and downstream signal proteins remain virtually unchanged.

In our view, the main disturbances in the AC system due to DM occur on the initial stages of signal transduction and are associated with changes in the expression of G proteins and hormonal receptors and violation of their functional interaction. There are reasons to regard the disturbances in hormone-regulated AC system, on the one hand, as a contribution in the development of dysfunctions of the cardiovascular, reproductive and other systems in DM leading to the complications of the disease and, on the other hand, as a compensatory response to physiological and biochemical changes occurring in a diabetic state [8,11]. It is known that the therapy of diabetic patients and experimental animals with insulin and other sugar-lowering drugs leads to normalization of the glucose level and restores the biochemical processes and physiological functions altered in DM. At the same time, the influence of the therapy on the functioning of hormonal signaling systems remains poorly studied and no information is available on the functional state of AC system in this case.

Insulin and its analogs with prolonged action find wide application in the treatment of insulin-sensitive DM, referred to as type 1 DM, as well as of insulin-independent DM, type 2 DM, especially at a later stage of this disease [13]. About 27% of diabetic patients continuously apply insulin for the management of their hyperglycemia [14]. In the recent years the method of insulin delivery by intranasal routes has been applied on a large scale. Intranasal insulin improves learning and memory, prevents cognitive decline, cerebral atrophy, and focal cerebral ischemia, and reduces food intake and body weight [15,16]. However, the molecular mechanisms and targets of intranasal insulin action have not been well defined. The involvement of hormonal signaling systems, AC system in particular, and their role in the intranasal insulin-mediated control of glucose homeostasis, energy metabolism, memory, and feeding behavior are not clear yet.