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Original article

Activity of the calcium-sensing receptor influences blood glucose and insulin levels in rats

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ABSTRACT

Background: The calcium-sensing receptor (CaR) has been found not only in parathyroid glands but also in other tissues, *e.g.* in β cells of the pancreatic islets. Therefore, CaR might likely mediate the mechanism of insulin secretion. The present study was designed to examine the *in vivo* effects of R-568, a CaR agonist, and NPS2143, a CaR inhibitor, on plasma insulin and blood glucose concentrations.
Methods: Wistar rats, after fasting for 14 h before the experiment, were anesthetized with inactin and loaded *ip* with 1 g/kg glucose.
Results: 20, 120 and 180 min after *iv* R-568 administration, plasma insulin increased markedly (by approximately 30%), in glucose-loaded rats, as compared to the control animals. **S**imultaneously, 180 min after R-568 administration, a significant drop by approximately 12% in blood glucose was observed. In contrast, administration of R-568 in rats not given glucose, did not influence the blood glucose or plasma insulin concentrations *vs.* the control group. Administration of NPS2143 increased the blood glucose level markedly (by about 18% *vs.* control group) at 180 and 210 min of the experiment. Simultaneously, a significant decrease of insulin concentration was observed *vs.* control group (by about 18 and 23%, respectively).
Conclusion: We suggest that modulation of the CaR activity may participate in the mechanisms which mediate insulin secretion in rats.

Introduction

The calcium-sensing receptor (CaR) belongs to the family of G protein-coupled and phospholipase C (PLC) activating receptors and plays a key role in regulation of plasma calcium concentration [1,2]. In addition to being expressed on the surface of parathyroid cells, CaR expression has been found in many other cells and tissues that are not directly involved in the systemic regulation of plasma Ca^{2+}, *e.g.* human aortic endothelial cells [3] fibroblasts [4], neuronal cells [5], breast ductal cells [6], or lens epithelial cells [7]. Recently, the presence of the CaR receptor in rat β cells of the pancreatic islets [8,9] and human insulinoma [10] has been reported.

Pancreatic β cells are involved in the process of insulin secretion. In response to increased plasma glucose concentration the intracellular ATP/ADP ratio rises, resulting in closure of the ATP-regulated K^+ channels and depolarization of the plasma membrane. This triggers opening of the voltage-dependent Ca^{2+} channels and subsequent rise of the free cytoplasmic Ca^{2+} which acts in multiple ways increasing the rate of exocytosis of insulin from the insulin storing secretory granules [11]. On the other hand, extracellular Ca^{2+} acts a cofactor activating the CaR, the receptor coupled with PLC enzyme. Activation of PLC leads to increase of cytoplasmic Ca^{2+} concentration due to liberation of Ca^{2+} from intracellular storages *via* diacylglycerol and phosphatidylinositol bisphosphate metabolic pathways [12]. Therefore, mediated by increased intracellular Ca^{2+} concentration, the rise in insulin secretion may be the effect of both high plasma glucose concentration, and CaR activation.

In addition to Ca^{2+}, the CaR may be activated by some other factors such as inorganic cations and organic polycations [13]. Calcimimetics such as phenylalkylamine R-568, activate CaR allosterically, which increases the affinity of the receptor for extracellular Ca^{2+} [14]. In the *in vitro* experiments performed on

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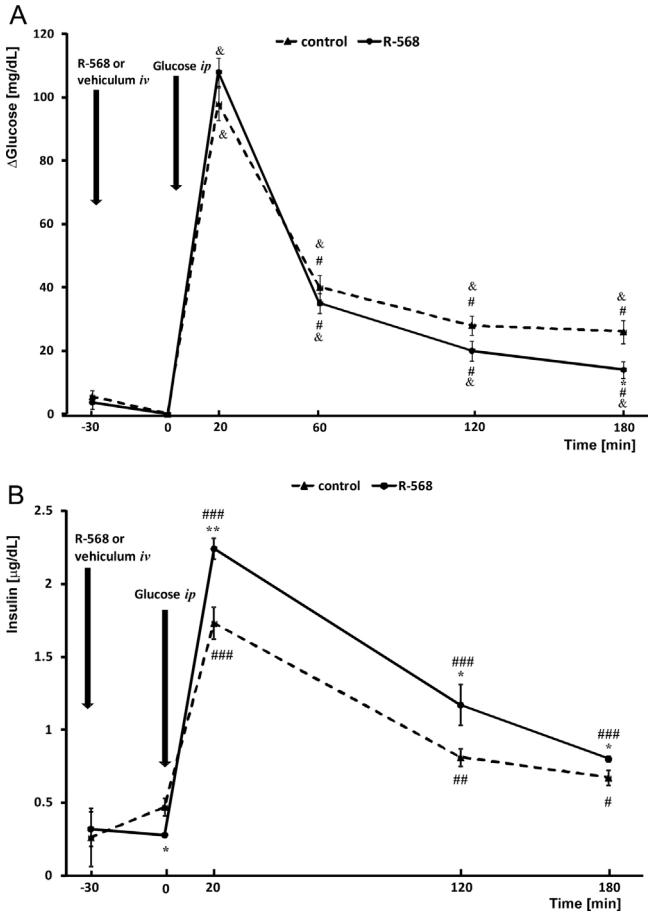

Fig. 1. Effect of *iv* R-568 or vehiculum administration on (**A**) Δ glucose calculated as the difference between sequential measurements of blood glucose concentration during experiment and concentration at the time 0 before *ip* glucose administration (control group n = 11; R-568 group n = 11); $^{*}p < 0.01$ *vs.* control group, $\#p < 0.001$ *vs.* time 20 min, $^{\&}p < 0.001$ *vs.* time 0; and (**B**) plasma insulin concentration (control group n = 4; R-568 group n = 4); $^{*}p < 0.05$, $^{**}p < 0.008$ *vs.* control group, $\#p < 0.04$, $\#\#p < 0.007$, $\#\#\#p < 0.001$ *vs.* time 0 in glucose-loaded rats. Comparisons were performed using Student's *t*-test.

pancreatic islets from C57BL/6 mice and on a mouse-derived βHC9 cell line, activation of the CaR by another agonist, R-467, increased insulin secretion in the presence of the stimulatory concentration of glucose [15]. Moreover, in the *in vitro* study where R-568 was used as the CaR activating agent, transient stimulation of insulin secretion from human islets of Langerhans was observed [16]. To date, there have been no studies showing whether activation of CaR in the *in vivo* conditions is effective in augmenting insulin secretion and decreasing blood glucose concentration. Furthermore, it is not known whether inhibition of the CaR by calcilytic NPS 2143 could provoke an opposite effect.

Therefore, in the present study we used *in vivo* models to determine the effect of the CaR agonist R-568, and CaR inhibitor NPS2143 on plasma insulin and blood glucose concentrations in rats.

Materials and methods

Animals and surgical procedures

Male Wistar rats weighing 250–280 g were purchased from the Animal House of the Medical University of Gdańsk (Gdańsk, Poland). The rats were kept at constant room temperature (20 °C) and humidity (70%), under the 12-h dark/light cycles. All experiments were approved by the Local Ethical Committee on Animal Experiments. The animals were fed commercial rodent chow (Labofeed-B, Warszawa, Poland) and provided with tap water *ad libitum*. Before the experiment the rats were fasted for 14 h (starting at 8 p.m.). On the day of experiment, the rats were anesthetized by intraperitoneal injection of inactin at the dose of 100 mg/kg b.w. The animals were placed on a heated table and their body temperature was maintained between 36 and 37 °C. Tracheostomy was performed, and catheters were inserted in the carotid artery for blood sampling, in the jugular vein for infusion, and in the bladder for free diuresis. After all surgical procedures, a 30 min recovery period was allowed to establish a steady state. Over the whole experiment, the rats were infused with isotonic saline supplemented with heparin (20 U/ml of the solution) at the rate of 1.2 ml/h.

Experimental groups

Effect of R-568 on blood glucose and Ca^{2+}, and on plasma insulin levels in glucose-loaded and normal rats

After the recovery period, R-568 dissolved in 15% cyclodextrin at the dose of 1 mg/kg b.w., was administered as a 100 μl bolus through the venous catheter. Thirty min later, glucose at the dose of 1 g/kg b.w. was given intraperitoneally. The time of glucose administration was designated as time 0 of the experiment. Glucose and Ca^{2+} measurements were performed before R-568 and glucose administration, and at 20, 60, 120, and180 min after glucose injection. Blood samples for insulin assay were taken before glucose injection, and 20, 120, and 180 min thereafter. Normal rats were treated identically, except that they received saline instead of glucose.

In the control groups, the animals were infused *iv* with 100 μl of 15% cyclodextrin instead of R-568. Otherwise, the experimental protocols were identical with that described above.

Effect of NPS 2143 on blood glucose and Ca^{2+}, and on plasma insulin levels in rats

After the recovery period, NPS 2143 dissolved in 15% cyclodextrin at the dose of 2 mg/kg b.w., was administered as a 100 μl bolus through the venous catheter. The time of NPS 2143 administration of was designated as time 0 of the experiment. Glucose and Ca^{2+} measurements were performed 10 min ahead of and immediately before the NPS 2143 injection, as well as 60, 120, 180, and 210 min thereafter. Blood samples for insulin assay were drawn 10 min before the NPS 2143 administration and 120, 180 and 210 min thereafter.

In the control group, the animals were infused with 100 μl of 15% cyclodextrin *iv* instead of the NPS 2143. Otherwise, the experimental protocol was identical with that described above.

Glucose, Ca^{2+} and insulin measurements

Blood glucose was measured using a glucometer (ACCU-CHECK Active, model: GC ACCU-CHEK, Roche, Mannheim, Germany). Ca^{2+} concentration in blood was measured using the 9180 Electrolyte Analyser, Roche Diagnostic GmbH, Mannheim, Germany. Insulin was determined in 10–25 μl plasma using an enzyme-linked immunosorbent assay DRG ultrasensitive Rat Insulin ELISA and DRG Insulin (Rat) ELISA (DRG International, Inc., Springfield, NJ, USA).

Drugs and chemicals

The following chemicals and drugs were used: Inactin hydrate CIII, Sigma, St. Louis, MO, USA; isotonic saline, Fresenius Kabi,