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Original article

Verapamil prevents the effect of calcium-sensing receptor activation on the blood glucose and insulin levels in rats

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ABSTRACT

Background: The Ca^{2+} triggered insulin exocytosis in β cells of the pancreatic islets may be the result of Ca^{2+} influx through L-type voltage dependent calcium channels (VDCC) localized in the plasma membrane, as well as of liberation of Ca^{2+} from intracellular storages, induced by activation of the calcium receptor (CaR) coupled with the PLC enzyme present in the pancreatic islets. The present study was designated to determine, in *in vivo* experiments, the effects of CaR activation by R-568 and inhibition of the receptor by NPS 2143 on the plasma glucose and insulin levels in the presence of verapamil, a calcium channel blocker.
Methods: Wistar rats, after fasting for 14 h before the experiment, were anesthetized with inactin and loaded *ip* with 1 g/kg glucose.
Results: In comparison to the control group, the verapamil-induced blockade of the calcium channels in glucose loaded animals increased the blood glucose level and decreased the insulin level, whereas CaR activation with R-568 induced opposite effects. However, in the presence of verapamil, R-568 did not change the concentration of glucose or insulin versus the control animals. Verapamil infusion did not alter elevated glucose concentration in the NPS 2143 animals. At the same time, verapamil reduced the plasma insulin level and potentiated the drop of insulin concentration induced by NPS 2143.
Conclusion: The observations suggest that under the *in vivo* conditions, calcium channel blockade may prevent changes in the blood glucose and insulin concentrations induced by the CaR activation.

Introduction

The pancreatic β-cells are involved in the process of insulin secretion. Several studies have revealed that the L-type voltage dependent calcium channels (VDCC) are expressed in islet β-cells and the insulin-secreting cell lines in any species [1,2] and play the predominant role in Ca^{2+} -triggered insulin exocytosis [2,3]. VDCC are located predominantly in the plasma membrane [4].

When plasma glucose level rises, pancreatic β-cells activate several intracellular signaling pathways including intracellular Ca^{2+} -signaling. In response to an 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 induces rapid conformational changes in the L-type VDCC, which switches the VDCC from a Ca^{2+} -impermeable to a highly permeable Ca^{2+} pore, and the latter allows extracellular calcium to enter the cytoplasm. The Ca^{2+} entry through the VDCC induces the secretory granules directly and triggers insulin secretion [4,5]. The activity and density of the β-cell VDCC are regulated by a variety of mechanisms, such as intracellular Ca^{2+} concentration, protein phosphorylation, translocation and interaction with other membrane proteins, all resulting in higher or lower levels of insulin exocytosis [6–9]. Abnormality of the β-cell VDCC can lead to β-cell impairment and β-cell death in diabetes of both type1 and type 2 [5,10].

Recent reports demonstrate that β cells of the pancreatic islets in rats [11,12] and human insulinoma [13] express the calcium sensing receptor (CaR). The CaR belongs to the family of G protein-coupled and phospholipase C (PLC) activating receptors. An activated PLC, by generating diacylglycerol and phosphatidylinositol bisphosphate, triggers the release of Ca^{2+} from intracellular stores, thus increasing Ca^{2+} concentration within the cell [14]. It seems likely that the mechanisms which underlie the rise of insulin secretion in response to elevated cytosolic Ca^{2+} could involve both opening of the voltage-dependent Ca^{2+} channels, and CaR activation. The CaR may be

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activated by Ca^{2+} and other factors such as inorganic cations, amino acids, organic polycations and polyamines [15]. Calcimimetics such as phenylalkylamine R-568, activate CaR allosterically, thus increasing the affinity of the receptor for extracellular Ca^{2+} [16]. Experiments performed *in vivo* suggest that modulation of CaR activity may also participate in the mechanisms which mediate insulin secretion. It has been shown that activation of the CaR with R-568 increased the plasma insulin concentration and decreased the glucose level in rats. Furthermore, adverse effects were observed after administration of the CaR inhibitor, NPS 2143, which decreased the insulin concentration and raised the blood glucose level [17]. These observations were corroborated in *in vitro* experiments with a mouse-derived βHC9 cell line [18] or human islets of Langerhans [19] where activation of the CaR by other agonists, R-467 or R-568, increased insulin secretion in the presence of a stimulatory concentration of glucose [18].

Therefore, the purpose of the present study was to determine, in *in vivo* experiments, how the blocking of the calcium channels by verapamil affects the changes in plasma glucose and insulin concentration induced by modification of the CaR activity, i.e. activation by the R-568 and inhibition by the NPS 2143.

Materials and methods

Animals and surgical procedures

Male Wistar rats weighing 250 to 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 (Bydgoszcz, Poland), Decision number 30/2018. 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 experiment day, the rats were anesthetized with an 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 at 36 to 37 °C. Tracheostomy was performed, and catheters were inserted: in the carotid artery for blood sampling and the monitoring of the mean arterial blood pressure (MAP), 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

Two basic experimental groups were established: rats administered glucose and not administered glucose.

The effect of R-568 on the blood levels of glucose, Ca^{2+}, insulin, and MAP in glucose-loaded rats in the presence of verapamil

The rats were divided into four experimental groups: those receiving verapamil and R-568 (n = 5), those receiving verapamil alone (n = 5), those receiving R-568 alone (n = 11), and the control group (n = 11). After the recovery period, infusion of verapamil at 8 μg/kg/min started. 10 min later, R-568 dissolved in 15% cyclodextrin at the dose of 1 mg/kg b.w. or 15% cyclodextrin alone were administered as 100 μl boluses through the venous catheter. After 30 min, glucose at the dose of 1 g/kg b.w. was given intraperitoneally. The time of glucose administration was set as time 0 of the experiment. Glucose, Ca^{2+}, and MAP measurements were performed before R-568 and glucose administration, and at 20, 60, 120, and180 min after glucose injection. Blood samples for an insulin assay were taken before glucose injection, and 20, 60, and 180 min thereafter.

In the verapamil group, 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.

In the control group, the animals did not receive verapamil infusion and were injected with 100 μl of 15% cyclodextrin *iv* instead of the R-568. Otherwise, the experimental protocol was identical with that described above.

The effect of R-568 or NPS 2143 on blood levels of glucose, Ca^{2+}, insulin, and MAP in normal rats not receiving glucose in the presence of verapamil

Experiments were performed on six groups of rats: those receiving verapamil and R-568 (n = 6) or NPS 2143 (n = 7), those receiving verapamil alone (n = 5), the ones receiving R-568 (n = 4) or NPS 2143 alone (n = 9), and the control group (n = 7). After the recovery period, infusion of verapamil 8 μg/kg/min started. 10 min later, R-568 at the dose 1 mg/kg b.w. or NPS 2143 at the dose of 2 mg/kg b.w., dissolved in 15% cyclodextrin, were administered as 100 μl boluses through the venous catheter. The time of R-568 or NPS 2143 administration was set as time 0 of the experiment. Glucose, Ca^{2+}, and MAP measurements were performed before the NPS 2143 or R-568 injection and 60, 120 and 180 min thereafter. Blood samples for an insulin assay were drawn 20 min prior and immediately before administration of the compounds, as well as 60, and 180 min thereafter.

In the verapamil group, the animals were infused with 100 μl of 15% cyclodextrin *iv* instead of R-568 or NPS 2143. Otherwise, the experimental protocol was identical with that described above.

In the control group, the animals did not receive a verapamil infusion and were injected with 100 μl of 15% cyclodextrin *iv* instead of R-568 or NPS 2143. Otherwise, the experimental protocol was identical with that described above.

Glucose, Ca^{2+}, insulin, and blood pressure 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). Arterial blood pressure was monitored directly using BIOPAC by Systems Inc., Model MP 100 (Goleta, CA, USA).

Drugs and chemicals

The following chemicals and drugs were used: Inactin hydrate CIII, Sigma, St. Louis, MO, USA; isotonic saline, Fresenius Kabi, Warszawa, Poland; Heparinum, POLFA, Warszawa, Poland. NPS 2143 and R-568 were purchased at TOCRIS, Tocris Bioscience, Bristol, UK. All other chemicals were purchased from Sigma-Aldrich, Poznań, Poland.

Statistical calculations

All values are presented as the mean ± SE. Comparisons were performed using the Student's *t*-test. The significance level was designated as $p < 0.05$.

Results

In glucose-loaded rats, the initial blood glucose concentration was 94 ± 2.0, 98 ± 5.1, 93 ± 2.8, and 96 ± 3.5, mg/dL in verapamil,

verapamil/R-568, R-568, and control groups, respectively. The effect of R-568 administration on blood glucose and plasma insulin concentration in the presence of verapamil in glucose-stimulated rats is shown in Fig. 1A. The blood glucose concentration is presented as the difference between sequential measurements during the experiment and the concentration at the time 0, before *ip* glucose administration. 20 min after glucose had been administered, its level rose similarly in all experimental groups as compared to the initial period. 60 min into the experiment, the decreases of blood glucose concentrations in the control, R-568, and verapamil/R-568 groups became more pronounced than in the verapamil rats. However, a significant difference starting at 60 min was observed only in the R-568 group, while in the verapamil/R-568 and control groups it was noted at 180 min into the experiment. Moreover, in glucose-loaded R-568 rats, blood glucose concentration was markedly lower than in the glucose-loaded verapamil/R-568 and control animals at 180 min of the experiment. Insulin concentration rose 20 min after the *ip* injection of glucose, then followed by a drop at 60 and 180 min in all animals. However, insulin rise observed in glucose-loaded R-568 rats was noticeably more prominent than in the other groups. The degree and the course of changes in insulin concentration in the control, verapamil, and verapamil/R-568 rats were similar, although in the verapamil group insulin concentration was the lowest and reached statistical significance *versus* the control group at 180 min of the experiment (Fig. 1B).

In normal animals not receiving glucose, the initial blood glucose concentrations were 89 ± 2.4, 96 ± 3.2, 98 ± 1.1, and 84 ± 1.5 mg/dL in the verapamil, verapamil/R-568, verapamil/NPS 2143, and control groups, respectively. The effect of the R-568 on blood glucose and plasma insulin concentration in the presence of verapamil is shown in Fig. 2. In the R-568, verapamil, and verapamil/R-568 animals no effect on blood glucose concentrations was observed in comparison to control group throughout the whole experiment (Fig. 2A). However, we observed a minor rise of glucose in the verapamil and verapamil/R-568 groups as compared

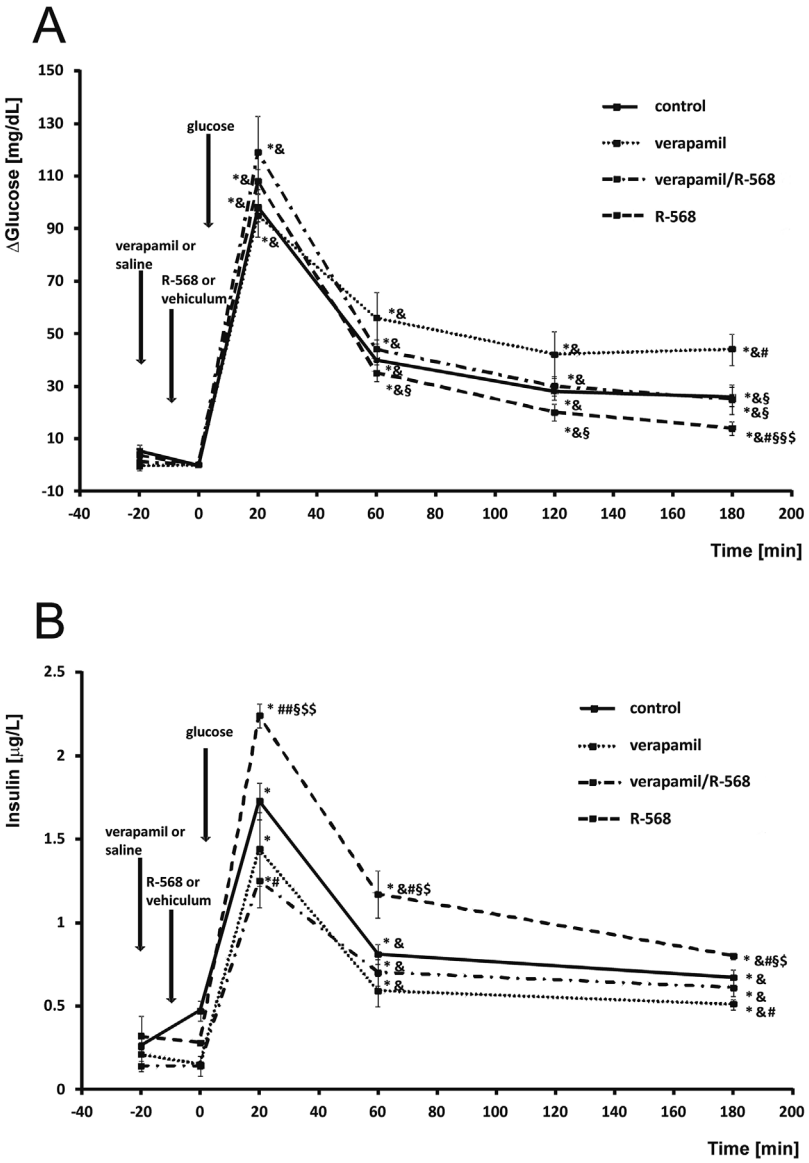

Fig. 1. Effect of *iv* R-568 or vehiculum administration on (**A**) Δ glucose calculated as the difference between sequential measurements of blood glucose concentration during experiment and concentration at the time 0 min before *ip* glucose administration (control n=11; verapamil n=5; verapamil/R-568 n=5; R-568 group n=11); *$p<0.001$ *vs.* time 0 min; &$p<0.001$ *vs.* time 20 min; §$p<0.01$, §§$p<0.001$ *vs.* verapamil; #$p<0.01$ *vs.* control; \$$p<0.05$ *vs.* verapamil/R-568 and (**B**) plasma insulin concentration (control n=4; verapamil n=4; verapamil/R-568 n=4; R-568 group n=4); *$p<0.001$ *vs.* time 0 min; &$p<0.001$ *vs.* time 20 min; #$p<0.05$, ##$p<0.008$ *vs.* control; §$p<0.01$ *vs.* verapamil; \$$p<0.01$, \$\$$p<0.001$ *vs.* verapamil/R-568 in glucose-loaded rats. Comparisons were performed using Student's *t*-test.

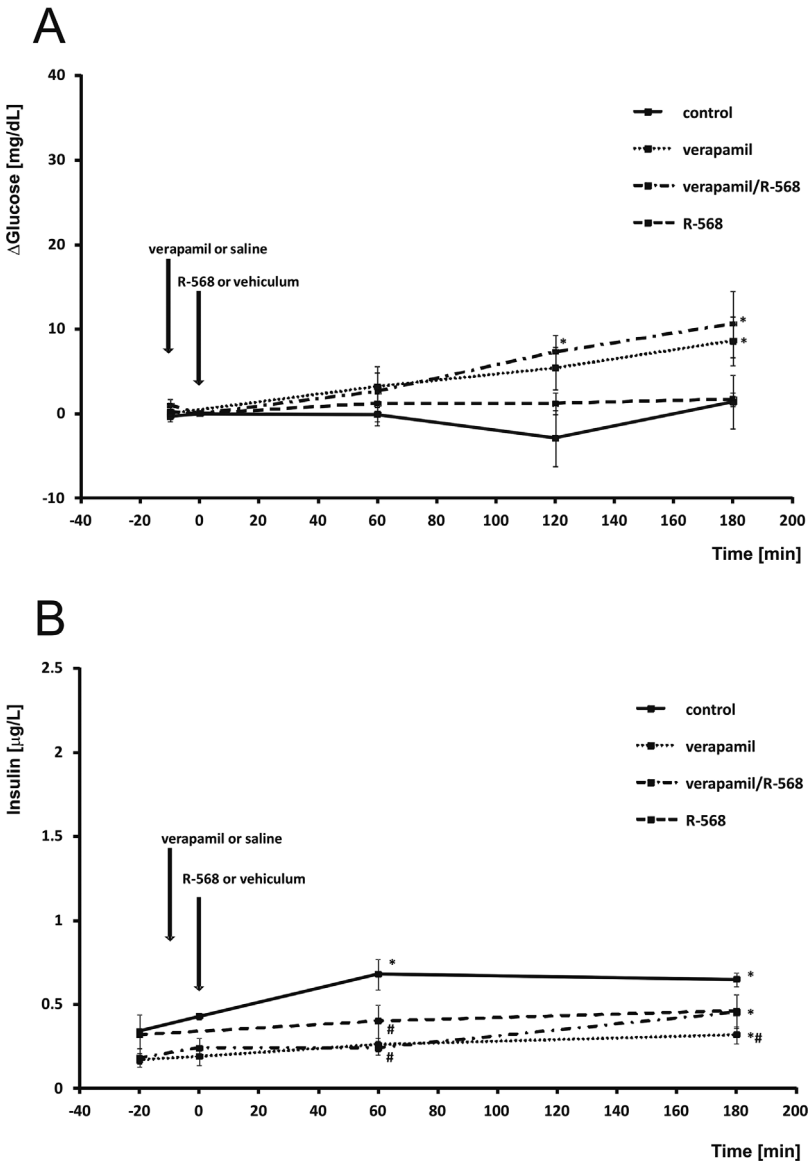

Fig. 2. Effect of *iv* R-568 or vehiculum administration on (**A**) Δ glucose calculated as the difference between sequential measurements of blood glucose concentration during experiment and concentration at the time 0 min before *iv* R-568 or vehiculum administration (control n=7; verapamil n=5; verapamil/R-568 n=6; R-568 group n=4); $^{*}p<0.05$ *vs.* time 0 min and (**B**) plasma insulin concentration (control n=4; verapamil n=4; verapamil/R-568 n=4; R-568 group n=4); $^{*}p<0.05$ *vs.* time 0 min; #$p<0.005$ *vs.* control group in normal, not loaded with glucose rats. Comparisons were performed using Student's *t*-test.

to the initial period. Plasma insulin level increased to a small extent in control, verapamil and verapamil/R-568 groups in comparison to initial period. In the verapamil group, plasma insulin was lower than in the control animals (Fig. 2B). Instead, in the NPS 2143 and verapamil/NPS2143 rats, similar and significant increases in glucose concentration *vs.* the control group and initial period were observed as of 120 min into the experiment (Fig. 3A). At 180 min of experiment, the increase reached the significance threshold *versus* the verapamil group. Simultaneously, marked drops of insulin concentration were observed in the NPS 2143, verapamil, and verapamil/NPS 2143 rats *vs.* the control group, and in the verapamil and verapamil/NPS 2143 groups *vs*. the NPS 2143 one (Fig. 3B).

In the normal and in the verapamil and/or glucose-loaded rats, administration of R-568 induced a transient decrease, while NPS 2143 injection induced a rise of the blood Ca^{2+} concentration in rats, as compared to the initial period and the control group (Table 1).

Infusion of verapamil provoked a modest decrease of blood pressure compared to the initial period, and the effect continued to the end of the experiment (Table 2).

Discussion

The results of this study suggest that in the *in vivo* conditions calcium channel blockade with verapamil prevents decrease of blood glucose and increase of insulin levels induced by R-568, calcium receptor (CaR) activator, in rats. Moreover, the presence of verapamil potentiates insulin decrease without affecting the elevated glucose concentration induced by inhibition of the CaR by NPS 2143.

It is well established that glucose metabolism in pancreatic β cells increases Ca^{2+} influx through the voltage-dependent Ca^{2+} channels (VDCC). An increased level of cytosolic free Ca^{2+} leads to insulin secretion. The release of Ca^{2+} from the endoplasmic reticulum, mediated by PLC-activated diacylglycerol and inositol triphosphate

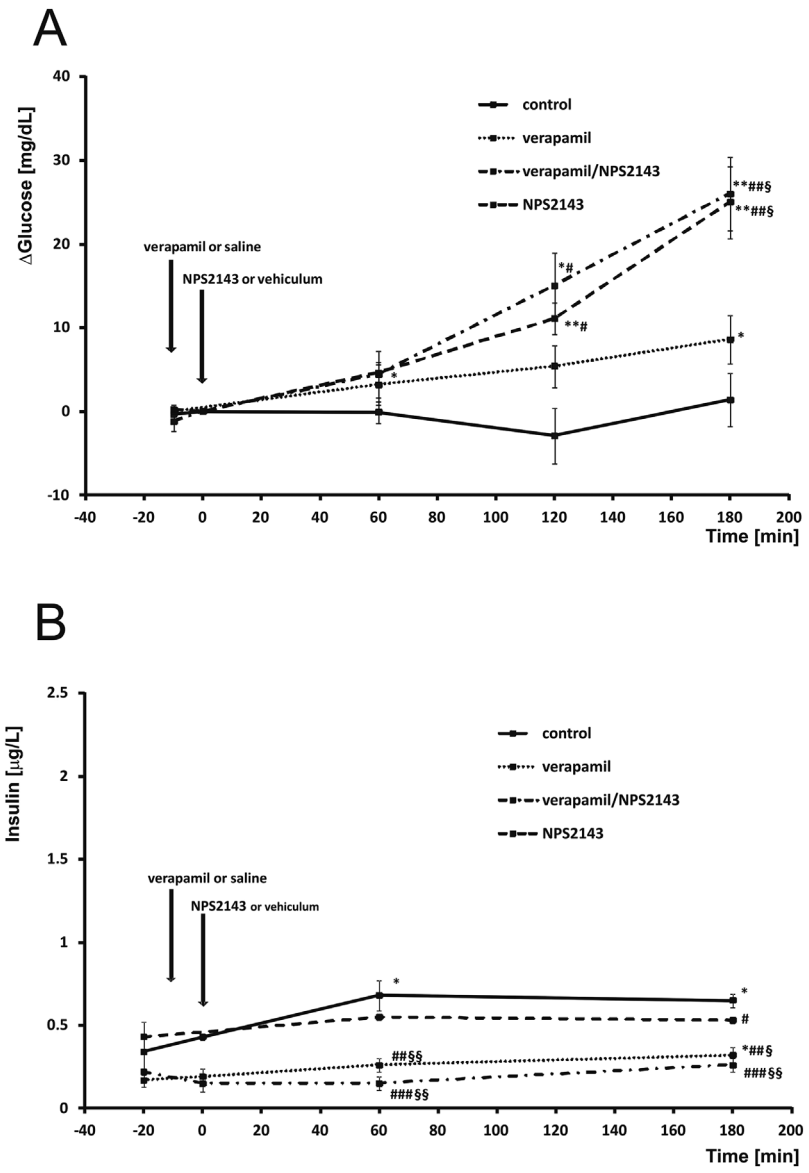

Fig. 3. Effect of *iv* NPS2143 or vehiculum administration on (**A**) Δ glucose calculated as the difference between sequential measurements of blood glucose concentration during experiment and concentration at the time 0 min before *iv* NPS2143 or vehiculum administration (control n=7; verapamil n=5; verapamil/NPS2143 n=7; NPS2143 group n=9); $^{*}p<0.05$, $^{**}p<0.001$ *vs.* time 0 min; #p<008, ##p<0.001 *vs.* control; §p<0.003 *vs.* verapamil and (**B**) plasma insulin concentration (control n=4; verapamil n=4; verapamil/NPS2143 n=4; NPS2143 group n=4); $^{*}p<0.05$ *vs.* time 0 min; #p<0.03, ##p<0.005, ###p<0.001 *vs.* control; §p<0.008, §§p<0.001 *vs.* NPS2143 group in rats. Comparisons were performed using Student's *t*-test.

pathways may also account for the elevation of intracellular Ca^{2+} concentration [5,20]. The latter pathway may be stimulated by activation of the CaR expressed on the surface of the pancreatic β cells [15]. This hypothesis was earlier supported by experiments performed *in vitro* on mouse pancreatic β cells C57BL/6 [18] and on human islets of Langerhans [19], where stimulation of insulin secretion by R-467 and R-568, agonists of the CaR, was demonstrated. Our recently published data derived from experiments *in vivo* confirmed the above observations indicating that activation of the CaR by R-568 increased plasma insulin concentration and decreased

Table 1
Effects of R-568 and NPS 2143 administration on blood Ca^{2+} concentration in normal and glucose loaded rats in the presence and absence of verapamil.

Time [min]	Ca^{2+} [mmol/L]									
	control (n = 7)	VER (n = 5)	VER/R-568 (n = 6)	VER/NPS 2143 (n = 7)	Glu (n = 6)	R-568/Glu (n = 4)	VER/ Glu (n = 5)	VER/R-568/Glu (n = 5)	R-568 (n = 4)	NPS 2143 (n = 4)
−10	1.36 ± 0.01	1.35 ± 0.01	1.37 ± 0.01	1.33 ± 0.01	1.37 ± 0.02	1.39 ± 0.02	1.36 ± 0.01	1.33 ± 0.01	1.31 ± 0.02	1.34 ± 0.02
60	1.36 ± 0.01	1.39 ± 0.02	1.19 ± 0.01*,#	1.44 ± 0.01*,#	1.39 ± 0.01	1.22 ± 0.03*,#	1.36 ± 0.01	1.19 ± 0.01*,#	1.18 ± 0.01*,#	1.44 ± 0.02*,#
120	1.37 ± 0.01	1.38 ± 0.02	1.22 ± 0.01*,#	1.44 ± 0.01*,#	1.39 ± 0.01	1.25 ± 0.01*,#	1.33 ± 0.01	1.19 ± 0.01*,#	1.24 ± 0.02*,#	1.46 ± 0.02*,#
180	1.37 ± 0.01	1.37 ± 0.02	1.25 ± 0.02*,#	1.38 ± 0.01*	1.37 ± 0.01	1.27 ± 0.01*,#	1.32 ± 0.02	1.22 ± 0.02*,#	1.30 ± 0.02#	1.42 ± 0.02*,#

VER, verapamil; Glu, glucose loaded; n, number of animals. Data are presented as means ± SE. Comparisons were made using Student's *t*-test: $^{*}p < 0.05$ *vs.* initial period, #$p < 0.05$ *vs.* control group.

Table 2
Effects of R-568 and NPS 2143 administration on mean arterial pressure in normal and glucose loaded rats in the presence and absence of verapamil.

Time [min]	MAP [mmHg]									
	control (n = 7)	VER (n = 5)	VER/R-568 (n = 6)	VER/NPS 2143 (n = 7)	Glu (n = 6)	R-568/Glu (n = 8)	VER/Glu (n = 5)	VER/R-568/Glu (n = 5)	R-568 (n = 4)	NPS 2143 (n = 9)
−10	120 ± 7	121 ± 5	119 ± 5	125 ± 7	118 ± 4	119 ± 7	127 ± 6	123 ± 7	122 ± 8	125 ± 8
0	112 ± 7	105 ± 6	105 ± 5	121 ± 7	110 ± 5	107 ± 8	112 ± 6	113 ± 8	110 ± 6	122 ± 6
60	108 ± 6	102 ± 4*	91 ± 5*	112 ± 7	107 ± 6	109 ± 5	112 ± 5	93 ± 7*	105 ± 7	111 ± 9
120	107 ± 7	102 ± 5*	103 ± 5*	109 ± 5*	105 ± 7	102 ± 6	106 ± 5*	94 ± 5*	106 ± 5	110 ± 6
180	109 ± 8	100 ± 4*	102 ± 4*	103 ± 4*	104 ± 8	100 ± 7	95 ± 4*	80 ± 10*	99 ± 8	107 ± 8

MAP, mean arterial blood pressure; VER, verapamil; Glu, glucose loaded; n, number of animals. Data are presented as means ± SE. Comparisons were made using Student's *t*-test: *$p<0.05$ *vs.* initial period.

blood glucose concentration in rats. Moreover, inhibition of the receptor with NPS 2143 resulted in a rise of glucose level and a decrease of insulin in rats [17]. The above results strongly suggest that the mechanisms mediating insulin secretion from the β islets may include modulation of the CaR activity.

In the present study using the *in vivo* model, we investigated the effect of verapamil blockade of the calcium channel on changes in glucose concentration and insulin secretion dependent on the CaR activity. Verapamil is known as an inhibitor of calcium influx through the L-type calcium channels [21], and an inhibitor of insulin release from the pancreatic islet cells [22], and as such it is used in the treatment of hypertension and severe supravetricular tachycardia [23].

Stable baselines of blood glucose and insulin levels were obtained by overnight fasting of the animals, similarly to the common procedures used in the published studies on glucose-loaded mice and rats [24,25].

Since R-568 administration did not influence insulin secretion or blood glucose concentration in non-loaded glucose animals [17], and activation of the CaR in *in vitro* experiments performed on β cells increased insulin secretion only in the presence of stimulatory glucose concentration [18], the investigation focused on the effect of the receptor activation in the presence of calcium channel blockade in *ip* glucose loaded rats. Intraperitoneal administration of glucose is widely accepted as a less stressful and easier alternative to oral gavage, it can also be more informative than any non-invasive route [25]. In glucose loaded animals, the verapamil blockade of the calcium channels increased blood glucose and decreased insulin levels *versus* the control group while CaR activation by way of R-568 administration induced the opposite effects. However, in the presence of verapamil, R-568 did not change the concentration of either glucose or insulin in comparison to the control animals (Fig. 1). These observations suggest that in the *in vivo* conditions, calcium channel blockade may prevent CaR-activation induced changes in the blood glucose and insulin concentrations. However, the increase in insulin concentration we observed in response to the R-568, along with similar maximal glucose levels in all groups of glucose loaded rats was considerably higher than in other experimental groups (Fig. 1). This may suggest that R-568 could induce insulin resistance. Further experiments are required to verify the hypothesis. Furthermore, in animals not loaded with glucose, R-568 administration in the presence of verapamil did not have an impact on the blood glucose concentration despite a modest drop of insulin level in the verapamil rats, as compared to the control group (Fig. 2).

Our previous observations revealed a rise of blood glucose and drop of insulin concentration in response to CaR inhibition in rats [17]. The present study confirmed the observation; moreover, it demonstrated the effect of CaR inhibition in the presence of calcium channel blockade. Verapamil itself did not change the blood glucose level, except for a minor rise *vs* initial period. Likewise, verapamil infusion did not influence the elevated glucose concentration in the NPS 2143 animals. Meanwhile, however, verapamil lowered the plasma insulin level and potentiated a drop in insulin concentration induced by NPS 2143 (Fig. 3).

It is well established that the CaR plays the key role in calcium homeostasis *via* parathormone (PTH) secretion [15]. In our earlier experiments, we confirmed that activation of the CaR with NPS R-568 decreases the plasma PTH and Ca^{2+} concentrations [26], while the receptor inhibition of with NPS 2143 enhanced both the PTH and Ca^{2+} levels [28]. Therefore, since the activity of the CaR is related to changes in the blood calcium concentration [15], it should be considered whether insulin secretion could be influenced by changes in the blood calcium level observed after administration of the R-568 and NPS 2143. However, the results we have published [26–28] and those reported by other authors [15,29] indicate that administration of R-568 and NPS 2143 in doses similar to those applied in the current study changes the blood calcium within the physiological range. The results of our present study corroborate the anticipated changes of the Ca^{2+} level in blood and the adequacy of the applied R-568 and NPS2143 doses. Moreover, verapamil alone was observed to have no effect on the blood calcium concentration (Table 1). The observation was also confirmed in the *in vitro* experiments performed on human islets, which showed that exposure to increasing Ca^{2+} concentrations (0.2–1.2 mM) did not affect insulin secretion [19]. Therefore, physiological fluctuations of the plasma Ca^{2+} concentration seem unlikely to modulate insulin secretion.

The mean arterial blood pressure was monitored throughout the experiment. As expected, in all groups of animals infused with the blocker, verapamil *per se* decreased the mean arterial blood pressure below the onset level. Furthermore, at the end of the experiment blood pressures were similar in all animal groups (Table 2).

In summary, the present study demonstrates that blockade of the calcium channels in the *in vivo* conditions prevents a rise of the plasma insulin and a decrease of the glucose level induced by CaR activation with R-568. On the other hand, calcium channel blockade did not alter the increased glucose but potentiated a drop in the insulin level induced by inhibition of the receptor.

Conflict of interests

No conflict of interest exists, including financial, personal or other relationships.

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