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The ablation of the $Ca_v2.3$/E-type voltage-gated Ca^{2+} channel causes a mild phenotype despite an altered glucose induced glucagon response in isolated islets of Langerhans

Alexey Pereverzev[a,b,1], Albert Salehi[d], Marina Mikhna[a], Erik Renström[d], Jürgen Hescheler[a,b], Marco Weiergräber[a,b], Neil Smyth[b,c], Toni Schneider[a,b,*]

[a]*Institute of Neurophysiology, University of Cologne, Robert-Koch-Str. 39, D-50931 Köln, Germany*
[b]*Center of Molecular Medicine Cologne, University of Cologne, Robert-Koch-Str. 39, D-50931 Köln, Germany*
[c]*Center for Biochemistry, Medical Faculty, University of Köln, D-50931 Köln, Germany*
[d]*Department of Molecular and Cellular Physiology, Department of Physiological Sciences, Lund University, Lund, Sweden*

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Abstract

Glucagon release upon hypoglycemia is an important homeostatic mechanism utilized by vertebrates to restore blood glucose to normal. Glucagon secretion itself is triggered by Ca^{2+} influx through voltage-gated ion channels, and the gene inactivation of R-type Ca^{2+} channels, with $Ca_v2.3$ as the ion conducting subunit, has been shown to disturb glucose homeostasis. To understand how glucagon release may be affected in $Ca_v2.3$-deficient mice, carbachol, insulin and glucose induced glucagon response was investigated. While the rise of insulin and glucose induced by carbachol is normal, mutant mice show an impaired glucagon-response. Further, the effect of insulin injection on glucagon levels was altered by the loss of the $Ca_v2.3$ subunit. $Ca_v2.3$-deficient mice are characterized by an impaired glucose suppression of glucagon release. This was most obvious at the level of isolated islets suggesting that $Ca_v2.3$ containing R-type voltage-gated Ca^{2+} channels are involved in the glucose-mediated signalling to glucagon release in mice.

Keywords: Islets of Langerhans; Peptide hormone-release; Cholinergic; Gene inactivation; Toxin-resistant current; R-type Ca^{2+} channel

1. Introduction

The release of peptide hormones is triggered by several voltage-gated Ca^{2+}-channels. For the release of insulin, the best known Ca^{2+} channels belong to the L-type Ca_v1 subfamily, as $Ca_v1.2$ (α1C) and $Ca_v1.3$ (α1D). To understand their contribution to insulin release, their genes were inactivated either in general or tissue specifically, leading only to a moderate endocrine phenotype (Hofmann et al., 1999; Namkung et al., 2001; Schulla et al., 2003).

In addition to the L-type family, another high voltage-gated subfamily, the Ca_v2 channels containing the ion conducting subunits of P/Q-, N- and R-type Ca^{2+}-channels (Mori et al., 1991; Seino et al., 1992; Williams et al., 1992, 1994; Schneider et al., 1994) are also involved in peptide hormone release (Catterall, 1999; Perez-Reyes, 2003). Even more, members of the recently discovered T-type subfamily Ca_v3 are expressed in endocrine systems, as for example $Ca_v3.2$ (Perez-Reyes, 2003; Satin, 2000; Leuranguer et al., 2000; Glassmeier et al., 2001; Schrier et al., 2001).

The R-type channels, sometimes also called E-type, contain $Ca_v2.3$ (α1E) as the ion conducting pore. This Ca^{2+}-channel subunit was cloned as neuronal (Williams et al.,

* Corresponding author. University of Cologne, Institute of Neurophysiology, Robert-Koch-Str. 39, D-50931 Köln, Germany. Tel.: +49 221 4786946; fax: +49 221 4786965.
E-mail address: Toni.Schneider@uni-koeln.de (T. Schneider).
[1] Present address: National Primate Research Center, The University of Wisconsin-Madison, Madison, WI 53715, USA.

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1994) and endocrine splice variants (Schneider et al., 1994; Pereverzev et al., 1998, 2002a) initially detected in the insulinoma cell line INS-1 (Vajna et al., 1998, 2001) and the heart (Weiergräber et al., 2000; Mitchell et al., 2002). The complex phenotype of $Ca_v2.3$ deficient mice reveals neuronal (Saegusa et al., 2000; Dietrich et al., 2003; Kubota et al., 2001; Lee et al., 2002) and endocrine deficits, resulting in a slight glucose intolerance and an impaired secretagogue induced insulin release (Matsuda et al., 2001; Pereverzev et al., 2002b).

Initial immunhistochemical investigations revealed to us that the glucagon releasing α cells of the islets of Langerhans also express $Ca_v2.3$ (Grabsch et al., 1999). Much less is known for the Ca^{2+} channels which trigger the release of glucagon. To further test the hypothesis that $Ca_v2.3$ is involved in the release of glucagon or in the modulation of its release, glucagon secretion from isolated islets as well as from whole animals was analyzed, after applying carbachol, insulin or glucose itself, in wild type mice and in mice lacking the $Ca_v2.3$ Ca^{2+} channel subunit.

2. Materials and methods

2.1. Materials

Carbachol (Sigma München, Germany) and human insulin Actrapid HM solution (40 I.U./ml; Novo Nordisk Pharma GmbH Mainz, Germany) were diluted in 0.9% NaCl sterile solution. Sterile glucose solution was from Delta Pharma GmbH (Pfullingen, Germany).

2.2. Animals and gene inactivation

Mice were housed at a constant temperature (22–23 °C), with light from 7 a.m. to 7 p.m. and ad libitum access to food and water. The animals were fed a breeding diet containing 22.5% crude protein, 5% crude fat, and 4.5% crude fiber (#1310, Altromin, Lage, Germany). The animal experimentation described in the text was approved by the institutional committee on animal care.

The *cacna1e* gene encoding $Ca_v2.3$ was disrupted by us in vivo by deleting a region containing exon 2 on mating $Ca_v2.3$(fl|+) and deleter-mice on a C57Bl/6 background (Pereverzev et al., 2002b) which express *Cre*-recombinase constitutively under the control of the CMV promoter (Schwenk et al., 1995). $Ca_v2.3$(+|−) mice containing one cre-transgene were inbred, and pups with the $Ca_v2.3$(−|−) genotype were selected and transferred by embryo transfer into a SPF facility. The transfer included the breeding with C57Bl/6 mice which delivered heterozygous $Ca_v2.3$(+|−) mice. Only cre-negative pups were selected and inbred yielding either $Ca_v2.3$(+|+) or $Ca_v2.3$(−|−) mice. Thus, parallel breeding ensured identical backgrounds between $Ca_v2.3$(+|+) and $Ca_v2.3$(−|−) mice.

2.3. Blood glucose, serum insulin and serum glucagon determination during insulin tolerance testing, and after injection of carbachol or glucose

Ten-week-old male mice were used. For glucagon determination, blood samples were collected from a small tail cut at the time intervals mentioned. Serum samples were prepared after blood clotting in a standard way by fast centrifugation. Glucagon in serum was measured by a rat/human glucagon, enzyme-linked immunosorbent assay (ELISA) kit (Yanaihara Institute Inc., Japan) according to the protocol of the manufacturer.

After carbachol injection, the serum glucagon and the blood glucose were determined at specific time points (Simonsson and Ahren, 1998). To test the influence of injected glucose on serum glucagon, 2 mg D-glucose/g body weight (Delta-Pharma GmbH) was injected intraperitonically. The blood glucose was determined in blood taken from the cut tail tip, before and after the administration of glucose at the times indicated in the figures. The glucose concentration was determined using the Glucometer Elite (Bayer Diagnostics GmbH, Germany).

Before experiments for insulin tolerance, mice were starved for 14 h but allowed free access to water. Insulin tolerance was tested by intraperitoneal injection of 0.75 international units human insulin Actrapid per kg body weight. The amount of insulin injected, was optimised in previous experiments. We used 0.75 I.U. which decreased glucose close to 50% of the initial values, about 1 hour after insulin injections (see Fig. 5). Serum insulin was measured in cut tail tip derived blood using the ELISA kit from Crystal Chem (Chicago, USA), after intraperitoneal injections of glucose, as well as carbachol or insulin.

2.4. Isolation of islets of Langerhans for glucagon release

Pancreatic islets were isolated by collagenase digestion as previously described (Schulla et al., 2003). Glucagon release was assayed in static batch incubations (10 islets per batch). The islets were first preincubated for 30 min at 37 °C in 1 ml in Krebs-Ringer bicarbonate buffer consisting of (in mM): 120 NaCl, 25 mM $NaHCO_3$, 4.7 KCl, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1 glucose and 10 Hepes (pH 7.4). The medium was gassed with 95% O_2–5% CO_2 to obtain constant pH and oxygenation. During the subsequent 60 min incubation period at 37 °C, the medium was changed to the test conditions indicated in text and figures. Immediately after incubation, a 25-μl aliquot of the medium was removed for assay of glucagon by radioimmunoassay (RIA).

2.5. Data analysis

Data are calculated and plotted throughout in the figures as the mean±standard error of the mean. Significance was estimated by *t*-test. Levels of $p<0.05$ were considered

statistically significant (*), and levels of $p<0.001$ as statistically highly significant (**).

3. Results

3.1. Glucagon release induced by carbachol is impaired in $Ca_v2.3(-|-)$ mice but its effect upon blood glucose is normal

Carbachol (Simonsson and Ahren, 1998) and insulin (Karlsson et al., 2002) stimulate glucagon release in mice. In our $Ca_v2.3(+|+)$ mice, the basal serum glucagon was similar as reported in other experiments with mice (Sund et al., 2001). Wild type and $Ca_v2.3$-deficient mice were always compared side by side, and in unstarved animals, intraperitoneal injection of carbachol (0.53 μmol/kg) raised serum glucagon in $Ca_v2.3(+|+)$-mice significantly from 411±16 pg/ml to 576±64 pg/ml (n=9; Fig. 1A), while in $Ca_v2.3(-|-)$ animals, the basal serum glucagon of 318±23 pg/ml was only slightly elevated to 339±28 pg/ml (n=8). These results suggest that the carbachol induced glucagon release is impaired in $Ca_v2.3$-deficient mice.

While carbachol is also known to effect insulin release, no significant alteration in the increase of insulin was seen 10 min after its administration, $Ca_v2.3(+|+)$ merely by 4.5 fold (n=7) and 5.6-fold (n=8) in $Ca_v2.3(-|-)$ mice (Fig. 1B).

Blood glucose was determined before and 10 min after i.p. injection of carbachol (Fig. 1C). It increased 1.33±0.07 and 1.31±0.07-fold in 9 $Ca_v2.3(+|+)$ and 10 $Ca_v2.3(-|-)$ mice, respectively, from 124±7 mg/dl to 166±14 mg/dl in wild type, and from 129±4 mg/dl to 168±19 mg/dl in $Ca_v2.3$ deficient mice. These results suggest that the blood glucose increase is similar in both genotypes but that 10 min after carbachol injection, the glucagon response is impaired in $Ca_v2.3$-deficient mice compared to $Ca_v2.3(+|+)$ animals.

3.2. Effect of insulin injection on serum glucagon, serum insulin and blood glucose in $Ca_v2.3(-|-)$ mice

Human insulin (2.0 I.U.) was also injected intraperitonically. As with carbachol, injected insulin caused a significant rise of serum glucagon in $Ca_v2.3(+|+)$ mice from 492±20 pg/ml to 568±26 (n=12) pg/ml. In $Ca_v2.3$-deficient mice, a milder and not significant increase was observed from 495±30 pg/ml to 548±43 (n=10) pg/ml (Fig. 2A) suggesting that the hypoglycemic stress induced glucagon release may be partially suppressed in $Ca_v2.3$-deficient mice.

After insulin injection in $Ca_v2.3(+|+)$ mice, serum insulin increased from 1716±88 pg/ml to 5478±536 pg/ml (n=11). Near identical results were recorded for $Ca_v2.3(-|-)$ mice, in which basal serum insulin increased from 1614±146 to 5883±557 pg/ml (n=10; Fig. 2B). Consecutively, blood glucose in $Ca_v2.3(+|+)$ mice dropped from 117±4 mg/dl to 61±3 mg/dl (n=12), and in $Ca_v2.3(-|-)$ mice from 127±5 mg/dl to 65±4 mg/dl (n=10; Fig. 2C), leading to the conclusion that the injection of a bolus of insulin does not impair the glucose response after 60 min in either genotype, but the insulin induced glucagon increase is attenuated in $Ca_v2.3$-deficient mice.

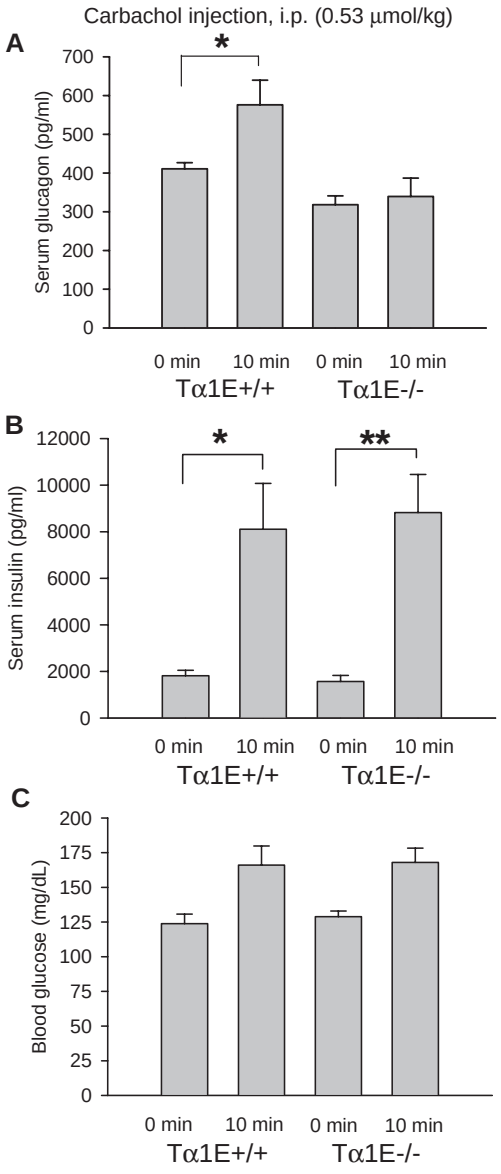

Fig. 1. Serum glucagon before and after carbachol injection. The male mice at the age of 10 weeks were fed ad libitum. (A) Serum glucagon before and 10 min after i.p. injection of 0.53 μmol/kg carbachol. Only in $Ca_v2.3(+|+)$ mice the increase after 10 min was significant (paired t-test, p=0.029; n=9), and not in $Ca_v2.3(-|-)$ mice (p=0.69; n=8). (B) Serum insulin before and 10 min after i.p. carbachol injection (10 wk old male mice). In $Ca_v2.3(+|+)$ mice, serum insulin was increased from 1817±235 pg/ml to 8105±1973 pg/ml (n=7), while in $Ca_v2.3(-|-)$ mice serum insulin was elevated from a basal value of 1571±261 pg/ml to 8824±1636 pg/ml (n=8). No significant difference was observed between the basal insulin of both genotypes (p=0.50), or the serum insulin after 10 min in between both groups (p=0.78). (C) Blood glucose before and 10 min after i.p. injection of carbachol (0.53 μmol/kg). The increase of glucose was similar in $Ca_v2.3(+|+)$ (n=9) and $Ca_v2.3(-|-)$ mice (n=10).

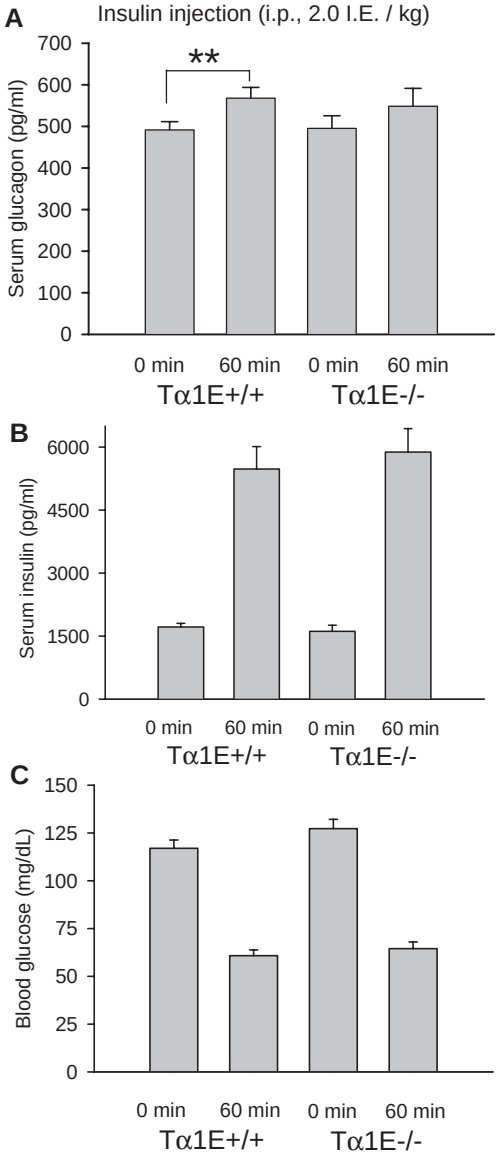

Fig. 2. Injection of insulin provokes similar responses in $Ca_v2.3(+|+)$ and $Ca_v2.3(-|-)$ mice. The data from 12 $Ca_v2.3(+|+)$ and 10 $Ca_v2.3$ deficient 10-week-old male mice are plotted. No fasting occurred. (A) Serum glucagon before and 60 min after intraperitoneal injection of 2.0 I.U./kg insulin. The increase of insulin from 12 $Ca_v2.3(+|+)$ mice was significant, but not for the $Ca_v2.3(-|-)$ animals. (B) Serum insulin before and 60 min after i.p. insulin injection. (C) Blood glucose before and 60 min after i.p. insulin injection.

3.3. Glucose induced suppression of glucagon release is impaired in islets from $Ca_v2.3$-deficient mice

To understand if the impaired glucagon response to glucose is caused directly by the deletion of $Ca_v2.3$ in the islets of Langerhans, and to understand its involvement in the disturbed glucose homeostasis of $Ca_v2.3$-deficient mice, islets were isolated and the glucose induced suppression of glucagon release was studied in isolated islets (Fig. 3A). In wild type mice, the increase of superfused glucose from 1 mM to 20 mM significantly suppressed glucagon release from 36±2 to 20±2 pg glucagon per islet per hour, while in $Ca_v2.3$ deficient mice, glucagon secretion was maintained at a rate of 43±4 and 42±5 pg/islet per hour, respectively. In conclusion, the suppression of glucagon release by elevated glucose is disturbed in isolated islets from $Ca_v2.3$-deficient mice.

Glucagon content of serum was determined also in whole mice after intraperitoneal injection of glucose (2 mg/g body weight). In ten $Ca_v2.3(+|+)$ control mice glucagon was reduced significantly from 971±49 pg/ml to 900±42 pg/ml (p=0.03; paired t-test), while in 6 $Ca_v2.3$ deficient mice only a very minor change from 718±84 pg/ml to 705±82 pg/ml was observed (Fig. 3B), confirming our conclusion drawn from isolated islets of Langerhans, that glucose suppresses less the release of glucagon in $Ca_v2.3$ deficient mice compared to control animals.

3.4. Body weight, basal glucose, insulin and glucagon levels

To understand if the observed defects in glucagon, insulin and glucose induced glucagon response cause metabolic disturbances, basal paramaters as body weight, blood glucose, and serum insulin as well as glucagon levels were determined. Thirtyone $Ca_v2.3(+|+)$ and 27 $Ca_v2.3(-|-)$

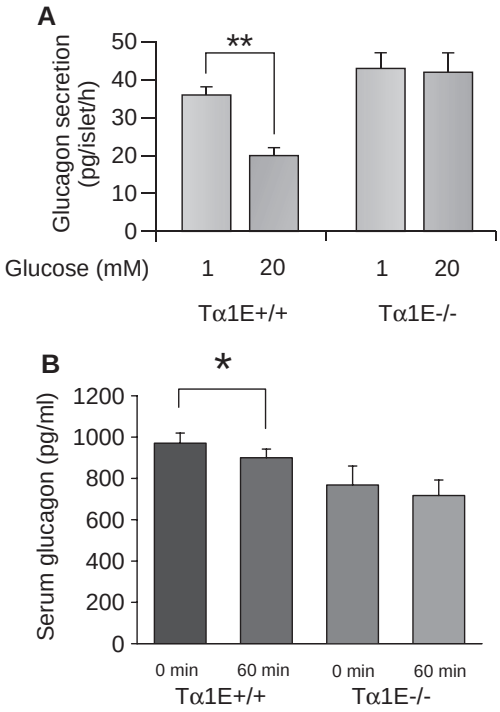

Fig. 3. Glucagon release in isolated islets and in mice. (A) Glucagon release in islets incubated at 1 or 20 mM glucose, in $Ca_v2.3(+|+)$ mice or $Ca_v2.3(-|-)$ mice, as indicated. Measurements were performed in 60-min static batch incubations (10 islets/batch) and data represent average values of 8 batches for each condition. (B) Determination of glucagon before and 60 min after intraperitoneal injection of glucose (2 mg/g body weight). In the paired t-test, the glucose induced suppression was significant in $Ca_v2.3(+|+)$ mice (n=10) but did not reach the level of significance in $Ca_v2.3$ deficient mice (n=6).

male mice were analysed for the basal values of body weight, blood glucose without starvation, insulin and glucagon levels. The mean body weight of the $Ca_v2.3(+|+)$ mice was 28.3 g±0.4 g, varying between 24.7 g and 35.8 g, while the $Ca_v2.3$-deficient mice were somewhat heavier and varied between 25.3 g and 36.1 g with a mean of 29.7 g±0.5 g (p=0.047) (Fig. 4A). The blood glucose of fed $Ca_v2.3$-deficient mice was significantly increased at 133±4 mg/dl (n=27) compared to $Ca_v2.3(+|+)$ control male at 123±3 mg/dl (n=31) (p=0.02) (Fig. 4B). Thus, body weight and blood glucose were slightly and significantly increased after inactivation of $Ca_v2.3$. For both, the serum insulin and the serum glucagon a slight difference was observed between both genotypes. Neither the serum insulin levels of 1664±109 pg/ml for $Ca_v2.3(+|+)$ and 1815±139 pg/ml for $Ca_v2.3(-|-)$ mice (Fig. 4C), nor the serum glucagon levels

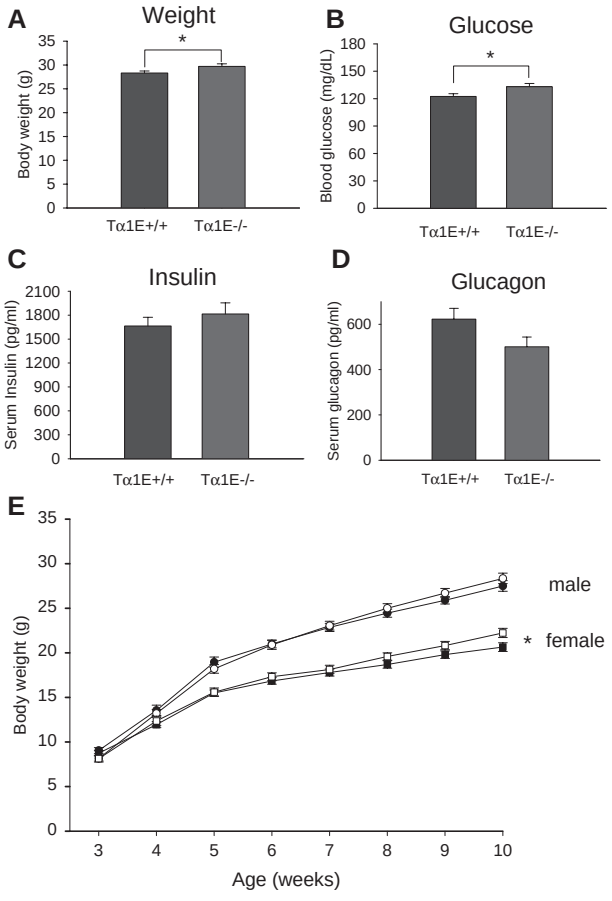

Fig. 4. Body weight, blood glucose, serum insulin, and serum glucagon from control and $Ca_v2.3$-deficient mice. The individual parameters were determined from mice which were not starved. $Ca_v2.3(+|+)$ mice, black columns, and $Ca_v2.3(-|-)$ mice, grey columns. (A) Body weight from 31 $Ca_v2.3(+|+)$ and 27 $Ca_v2.3(-|-)$ mice. (B) Blood glucose from 31 $Ca_v2.3(+|+)$ and 27 $Ca_v2.3(-|-)$ mice. (C) Serum insulin was determined in an ELISA test using rat insulin as a standard and were derived from 30 $Ca_v2.3(+|+)$ and 27 $Ca_v2.3(-|-)$ mice. The insulin increase does not reach the level of significance. (D) Serum glucagon was determined using an ELISA test. The values were derived from 31 $Ca_v2.3(+|+)$ and 27 $Ca_v2.3(-|-)$ mice. The glucagon reduction does not reach the level of significance. (E) Body weight of male (n=20) and female mice (n=22).

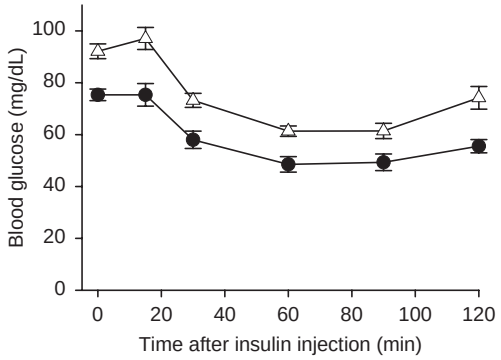

Fig. 5. Insulin tolerance test of $Ca_v2.3(+|+)$- and $Ca_v2.3$-deficient male mice. Blood glucose content during 120 min tolerance testing period after the intraperitoneal injection of 0.75 international units (I.U.) of human insulin per kg body weight in 10–12 week old male mice. Mean values of 7 $Ca_v2.3(+|+)$ (●) and 14 $Ca_v2.3(-|-)$ mice (△).

with 623±48 pg/ml ($Ca_v2.3(+|+)$) and 501±43 pg/ml ($Ca_v2.3(-|-)$) mice (Fig. 4D) were significantly different upon an ad libitum diet.

The more systematic investigation of the body weights showed a slight increase in growth rate for $Ca_v2.3$-deficient mice between 3 and 10 weeks of age (Fig. 4E). In conclusion, $Ca_v2.3$ deficient mice have a minor increase in blood glucose content and a small increase of their body weight (Fig. 4A and F). Basal insulin increase and glucagon decrease might be part of a compensation mechanism.

3.5. Testing of insulin tolerance in $Ca_v2.3$-deficient mice

To investigate whether insulin action is affected in $Ca_v2.3$ deficient mice, an insulin tolerance test was performed. After starvation, the blood glucose in paired $Ca_v2.3(+|+)$ and $Ca_v2.3(-|-)$ mice was compared during an insulin challenge. This revealed a significant increase of blood glucose in $Ca_v2.3$-deficient mice (Fig. 5). However, the normalized blood glucose, plotted as percentage of glucose before insulin injection does not differ between both genotypes during the whole tolerance test. Thus, the insulin stimulated glucose uptake is unaffected by the absence of the $Ca_v2.3$ channel.

4. Discussion

Glucagon is one of the major counter-regulatory hormones for insulin. We investigated the glucagon release in $Ca_v2.3$ deficient mice by intraperitoneal injection of carbachol and insulin, and the suppression of glucagon release by glucose in both, isolated islets and whole animals. To exclude other genetic influences, animals on identical backgrounds were used. The most important finding of the present study is the observation that isolated islets from $Ca_v2.3$ deficient mice do not reduce their glucagon release at elevated glucose levels. Also during testing of glucose tolerance in mice, the glucagon level in serum was not

significantly reduced after intraperitoneal injection of glucose in $Ca_v2.3$ deficient animals. However, the glucose induced glucagon suppression was normal in control mice.

Further, carbachol or insulin induced glucagon release is impaired in $Ca_v2.3$-deficient mice. As glucose induced insulin release is reduced (Pereverzev et al., 2002b) and as glucagon release is not increased but impaired, one would expect as reported for diabetic humans (Shah et al., 2000) additive effects of both disturbances. Glucose would be consumed more slowly by the different organs, and still be released from the glycogen storage. However, glucose tolerance after intraperitoneal injection is very similar (Pereverzev et al., 2002b) between animals of both genotypes.

These observations can only partially be explained by the known mechanism of glucagon release from α cells (Ashcroft, 2000; Barg et al., 2000; Gromada et al., 2001). As in β cells, the hormone secretion in α cells is triggered by Ca^{2+} influx through voltage-gated Ca^{2+} channels (Barg, 2003). The α cells contain a prominent tetrodotoxin-sensitive Na^+ channel, and at least 2 types of high voltage gated Ca^{2+} channels (Barg et al., 2000). Further, in guinea pigs a low voltage gated T-type current has also been described (Rorsman, 1988). After blocking N- and L-type voltage gated Ca^{2+} channels, up to 31% of an R-type like current is left in α cells of mice (Barg et al., 2000). Hence, one might speculate, that $Ca_v2.3$-containing R-type Ca^{2+} channels could constitute part of this component, and even more, the contribution of R-type inward currents might be under estimated, because $Ca_v2.3$ induced currents are partially sensitive towards dihydropyridines (Stephens et al., 1997).

Both, α cells and β cells are electrically excitable and generate Na^+ and Ca^{2+} dependent action potentials. Both cell types show an inverse glucose dependence for hormone secretion. Indeed, in both hormone release cascades, ATP-dependent K^+ channels are involved and initiate signalling (Ashcroft, 2000). However in α, unlike in β cells, the activity of K_{ATP}-channels is low. In α cells, increased glucose levels suppress glucagon secretion by inducing the closure of remaining K_{ATP}-channels. This leads to further membrane depolarisation, inactivating the ion channels which participate in action potential generation (Göpel et al., 2000). If $Ca_v2.3$-containing R-type Ca^{2+} channels are involved in depolarising the α cells in response to increased glucose levels, this might explain the lack of suppression of glucagon release at elevated (20 mM) glucose in isolated $Ca_v2.3(-|-)$ islet cells. As glucose tolerance is unchanged, additional factors involved in glucose homeostasis other than insulin and glucagon (possibly adrenaline, noradrenaline, cortisol, somatostatin or growth hormone), must contribute to the adjusted levels of blood glucose in $Ca_v2.3$ deficient mice. For example, the release of adrenaline from murine adrenal glands is dependent on R-type Ca^{2+} channels, resulting in a reduced stress induced hyperglycemia in $Ca_v2.3$ deficient mice (Albillos et al., 2000; Pereverzev et al., 2002b).

Also, it cannot be excluded that $Ca_v2.3$ containing R-type Ca^{2+} channels trigger somatostatin release from γ cells resulting in an impaired glucose-induced, and somatostatin-mediated glucagon-suppression in the $Ca_v2.3$-deficient mice.

We tested the hypothesis that $Ca_v2.3$-containing R-type Ca^{2+} channels are involved in the carbachol induced glucagon release. This stimulation is routinely used for increasing glucagon as well as insulin release in mice (Simonsson and Ahren, 1998). The effect of carbachol or acetylcholine on insulin release (Satin and Kinard, 1998) is better understood than its effect on glucagon release. In pancreatic β-cells acetylcholine binds to muscarinic M_3 receptors and exerts different effects including the stimulation of glucose-induced insulin release (for review: (Gilon and Henquin, 2001). Acetylcholine may increase cytosolic Ca^{2+} in the pancreatic β-cells in a number of ways, either by mobilization of intracellular stores, by capacitative Ca^{2+} entry, or by Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Gilon and Henquin, 2001) through the activation of protein kinase C (Tang and Sharp, 1998). The depolarization produced by acetylcholine also triggers Ca^{2+} influx through voltage-dependent Ca^{2+} channels at near-stimulating glucose concentrations (at 6–7 mM) (Gilon and Henquin, 2001).

Much less is known for the acetylcholine mediated effects on the pancreatic α-cells, but recently the gene inactivation of the muscarinic M_3 receptor in mice showed that both, pancreatic insulin and glucagon release are impaired (Duttaroy et al., 2004). Our present study shows that $Ca_v2.3$ containing R-type Ca^{2+} channels slightly affect glucagon release in vivo, but appear more important in vitro, by supporting the glucose mediated suppression of glucagon release as observed in isolated islets. In vivo, one must deduce, that the muscarinic stimulation of glucagon release and maybe also insulin release is triggered by $Ca_v2.3$ containing E-type Ca^{2+} channels.

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References

Albillos, A., Neher, E., Moser, T., 2000. R-type Ca^{2+} channels are coupled to the rapid component of secretion in mouse adrenal slice chromaffin cells. J. Neurosci. 20, 8323–8330.

Ashcroft, F.M., 2000. The Yin and Yang of the K_{ATP} channel. J. Physiol. 528, 405.

Barg, S., 2003. Mechanisms of exocytosis in insulin-secreting B-cells and glucagon-secreting A-cells. Pharmacol. Toxicol. 92, 3–13.

Barg, S., Galvanovskis, J., Göpel, S.O., Rorsman, P., Eliasson, L., 2000. Tight coupling between electrical activity and exocytosis in mouse glucagon-secreting a-cells. Diabetes 49, 1500–1510.

Catterall, W.A., 1999. Interactions of presynaptic Ca^{2+} channels and snare proteins in neurotransmitter release. Ann. N.Y. Acad. Sci. 868, 144–159.

Dietrich, D., Kirschstein, T., Kukley, M., Pereverzev, A., von der Brelie, C., Schneider, T., Beck, H., 2003. Functional specialization of presynaptic Cav2.3 Ca^{2+} channels. Neuron 39, 483–496.

Duttaroy, A., Zimliki, C.L., Gautam, D., Cui, Y., Mears, D., Wess, J., 2004. Muscarinic stimulation of pancreatic insulin and glucagon release is abolished in M3 muscarinic acetylcholine receptor-deficient mice. Diabetes 53, 1714–1720.

Gilon, P., Henquin, J.C., 2001. Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. Endocr. Rev. 22, 565–604.

Glassmeier, G., Hauber, M., Wulfsen, I., Weinsberg, F., Bauer, C.K., Schwarz, J.R., 2001. Ca^{2+} channels in clonal rat anterior pituitary cells (GH_3/B_6). Pflugers Arch. 442, 577–587.

Göpel, S.O., Kanno, T., Barg, S., Weng, X.G., Gromada, J., Rorsman, P., 2000. Regulation of glucagon release in mouse α-cells by K_{ATP} channels and inactivation of TTX-sensitive Na^{+} channels. J. Physiol. (Lond.) 528, 509–520.

Grabsch, H., Pereverzev, A., Weiergräber, M., Schramm, M., Henry, M., Vajna, R., Beattie, R.E., Volsen, S.G., Klöckner, U., Hescheler, J., Schneider, T., 1999. Immunohistochemical detection of α1E voltage-gated Ca^{2+} channel isoforms in cerebellum, INS-1 cells, and neuroendocrine cells of the digestive system. J. Histochem. Cytochem. 47, 981–993.

Gromada, J., Hoy, M., Buschard, K., Salehi, A., Rorsman, P., 2001. Somatostatin inhibits exocytosis in rat pancreatic a-cells by G_{i2}-dependent activation of calcineurin and depriming of secretory granules. J. Physiol. 535, 519–532.

Hofmann, F., Lacinova, L., Klugbauer, N., 1999. Voltage-dependent calcium channels: from structure to function. Rev. Physiol., Biochem. Pharmacol. 139, 33–87.

Karlsson, S., Scheurink, A.J., Ahren, B., 2002. Gender difference in the glucagon response to glucopenic stress in mice. Am. J. Physiol., Regul. Integr. Comp. Physiol. 282, R281–R288.

Kubota, M., Murakoshi, T., Saegusa, H., Kazuno, A., Zong, S., Hu, Q., Noda, T., Tanabe, T., 2001. Intact LTP and fear memory but impaired spatial memory in mice lacking Cav2.3 (α1E) channel. Biochem. Biophys. Res. Commun. 282, 242–248.

Lee, S.C., Choi, S., Lee, T., Kim, H.L., Chin, H., Shin, H.S., 2002. Molecular basis of R-type calcium channels in central amygdala neurons of the mouse. Proc. Natl. Acad. Sci. U. S. A. 99, 3276–3281.

Leuranguer, V., Monteil, A., Bourinet, E., Dayanithi, G., Nargeot, J., 2000. T-type calcium currents in rat cardiomyocytes during postnatal development: contribution to hormone secretion. Am. J. Physiol., Heart Circ. Physiol. 279, H2540–H2548.

Matsuda, Y., Saegusa, H., Zong, S., Noda, T., Tanabe, T., 2001. Mice lacking $Ca_v2.3$ (α1E) calcium channel exhibit hyperglycemia. Biochem. Biophys. Res. Commun. 289, 791–795.

Mitchell, J.W., Larsen, J.K., Best, P.M., 2002. Identification of the calcium channel alpha 1E (Ca(v)2.3) isoform expressed in atrial myocytes. Biochim. Biophys. Acta 1577, 17–26.

Mori, Y., Friedrich, T., Kim, M.-S., Mikami, A., Nakai, J., Ruth, P., Bosse, E., Hofmann, F., Flockerzi, V., Furuichi, T., Mikoshiba, K., Imoto, K., Tanabe, T., Numa, S., 1991. Primary structure and functional expression from complementary DNA of a brain calcium channel. Nature 350, 398–402.

Namkung, Y., Skrypnyk, N., Jeong, M.J., Lee, T., Lee, M.S., Kim, H.L., Chin, H., Suh, P.G., Kim, S.S., Shin, H.S., 2001. Requirement for the L-type Ca(2+) channel alpha(1D) subunit in postnatal pancreatic beta cell generation. J. Clin. Invest. 108, 1015–1022.

Pereverzev, A., Klöckner, U., Henry, M., Grabsch, H., Vajna, R., Olyschläger, S., Viatchenko-Karpinski, S., Schröder, R., Hescheler, J., Schneider, T., 1998. Structural diversity of the voltage-dependent Ca^{2+} channel α1E subunit. Eur. J. Neurosci. 10, 916–925.

Pereverzev, A., Leroy, J., Krieger, A., Malecot, C.O., Hescheler, J., Pfitzer, G., Klockner, U., Schneider, T., 2002. Alternate splicing in the cytosolic II–III loop and the carboxy terminus of human e-type voltage-gated Ca^{2+} channels: electrophysiological characterization of isoforms. Mol. Cell. Neurosci. 21, 352–365.

Pereverzev, A., Mikhna, M., Vajna, R., Gissel, C., Henry, M., Weiergräber, M., Hescheler, J., Smyth, N., Schneider, T., 2002. Disturbances in glucose-tolerance, insulin-release and stress-induced hyperglycemia upon disruption of the $Ca_v2.3$ (α1E) subunit of voltage-gated Ca^{2+} channels. Mol. Endocrinol. 16, 884–895.

Perez-Reyes, E., 2003. Molecular physiology of low-voltage-activated T-type calcium channels. Physiol. Rev. 83, 117–161.

Rorsman, P., 1988. Two types of Ca2+ currents with different sensitivities to organic Ca^{2+} channel antagonists in guinea pig pancreatic alpha 2 cells. J. Gen. Physiol. 91, 243–254.

Saegusa, H., Kurihara, T., Zong, S.Q., Minowa, O., Kazuno, A.A., Han, W.H., Matsuda, Y., Yamanaka, H., Osanai, M., Noda, T., Tanabe, T., 2000. Altered pain responses in mice lacking α1E subunit of the voltage-dependent Ca^{2+} channel. Proc. Natl. Acad. Sci. U. S. A. 97, 6132–6137.

Satin, L.S., 2000. Localized calcium influx in panreatic β-cells. Endocrine 13, 251–262.

Satin, L.S., Kinard, T.A., 1998. Neurotransmitter and their receptors in the islets of langerhans of the pancreas. Endocrine 8, 213–223.

Schneider, T., Wei, X., Olcese, R., Costantin, J.L., Neely, A., Palade, P., Perez-Reyes, E., Qin, N., Zhou, J., Crawford, G.D., Smith, R.G., Appel, S.H., Stefani, E., Birnbaumer, L., 1994. Molecular analysis and functional expression of the human type E α1 subunit. Recept. Channels 2, 255–270.

Schrier, A.D., Wang, H., Talley, E.M., Perez-Reyes, E., Barrett, P.Q., 2001. alpha1H T-type Ca2+ channel is the predominant subtype expressed in bovine and rat zona glomerulosa. Am. J. Physiol., Cell Physiol. 280, C265–C272.

Schulla, V., Renstrom, E., Feil, R., Feil, S., Franklin, I., Gjinovci, A., Jing, X.J., Laux, D., Lundquist, I., Magnuson, M.A., Obermuller, S., Olofsson, C.S., Salehi, A., Wendt, A., Klugbauer, N., Wollheim, C.B., Rorsman, P., Hofmann, F., 2003. Impaired insulin secretion and glucose tolerance in beta cell-selective Ca(v)1.2 Ca2+ channel null mice. EMBO J. 22, 3844–3854.

Schwenk, F., Baron, U., Rajewsky, K., 1995. A cre-transgenic mouse strain for the ubiquitous deletion of loxP-flanked gene segments including deletion in germ cells. Nucleic Acids Res. 23, 5080–5081.

Seino, S., Chen, L., Seino, M., Blondel, O., Takeda, J., Johnson, J.H., Bell, G.I., 1992. Cloning of the α_1 subunit of a voltage-dependent calcium channel expressed in pancreatic β cells. Proc. Natl. Acad. Sci. U. S. A. 89, 584–588.

Shah, P., Vella, A., Basu, A., Basu, R., Schwenk, W.F., Rizza, R.A., 2000. Lack of suppression of glucagon contributes to postprandial hyperglycemia in subjects with type 2 diabetes mellitus. J. Clin. Endocrinol. Metab. 85, 4053–4059.

Simonsson, E., Ahren, B., 1998. Potentiated beta-cell response to non-glucose stimuli in insulin-resistant C57BL/6J mice. Eur. J. Pharmacol. 350, 243–250.

Stephens, G.J., Page, K.M., Burley, J.R., Berrow, N.S., Dolphin, A.C., 1997. Functional expression of rat brain cloned alpha1E calcium channels in COS-7 cells. Pflugers Arch. 433, 523–532.

Sund, N.J., Vatamaniuk, M.Z., Casey, M., Ang, S.L., Magnuson, M.A., Stoffers, D.A., Matschinsky, F.M., Kaestner, K.H., 2001. Tissue-specific deletion of Foxa2 in pancreatic beta cells results in hyperinsulinemic hypoglycemia. Genes Dev. 15, 1706–1715.

Tang, S.H., Sharp, G.W., 1998. Atypical protein kinase C isozyme zeta mediates carbachol-stimulated insulin secretion in RINm5F cells. Diabetes 47, 905–912.

Vajna, R., Schramm, M., Pereverzev, A., Arnhold, S., Grabsch, H., Klöckner, U., Perez-Reyes, E., Hescheler, J., Schneider, T., 1998. New isoform of the neuronal Ca^{2+} channel α1E subunit in islets of Langerhans, and kidney. Distribution of voltage-gated Ca^{2+} channel α1 subunits in cell lines and tissues. Eur. J. Biochem. 257, 274–285.

Vajna, R., Klöckner, U., Pereverzev, A., Weiergräber, M., Chen, X.H., Miljanich, G., Klugbauer, N., Hescheler, J., Perez-Reyes, E., Schneider, T., 2001. Functional coupling between 'R-type' calcium channels and insulin secretion in the insulinoma cell line INS-1. Eur. J. Biochem. 268, 1066–1075.

Weiergräber, M., Pereverzev, A., Vajna, R., Henry, M., Schramm, M., Nastainczyk, W., Grabsch, H., Schneider, T., 2000. Immunodetection of α1E voltage-gated Ca2+ channel in chromogranin-positive muscle cells of rat heart, and in distal tubules of human kidney. J. Histochem. Cytochem. 48, 807–819.

Williams, M.E., Feldman, D.H., McCue, A.F., Brenner, R., Velicelebi, G., Ellis, S.B., Harpold, M.M., 1992. Structure and functional expression of α_1, α_2, and β subunits of a novel human neuronal calcium channel subtype. Neuron 8, 71–84.

Williams, M.E., Marubio, L.M., Deal, C.R., Hans, M., Brust, P.F., Philipson, L.H., Miller, R.J., Johnson, E.C., Harpold, M.M., Ellis, S.B., 1994. Structure and functional characterization of neuronal α1E calcium channel subtypes. J. Biol. Chem. 269, 22347–22357.