Effect of Neonatal Streptozotocin and Thyrotropin-Releasing Hormone Treatments on Insulin Secretion in Adult Rats

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Abstract. Neonatal STZ (nSTZ) treatment results in damage of pancreatic B-cells and in parallel depletion of insulin and TRH in the rat pancreas. The injury of B-cells is followed by spontaneous regeneration but dysregulation of the insulin response to glucose persists for the rest of life. Similar disturbance in insulin secretion was observed in mice with targeted TRH gene disruption. The aim of present study was to determine the role of the absence of pancreatic TRH during the perinatal period in the nSTZ model of impaired insulin secretion. Neonatal rats were injected with STZ (90 μg/g BW i.p.) and the effect of exogenous TRH (10 ng/g BW/day s.c. during the first week of life) on *in vitro* functions of pancreatic islets was studied at the age 12–14 weeks. RT-PCR was used for determination of prepro-TRH mRNA in isolated islets. Plasma was assayed for glucose and insulin, and isolated islets were used for determination of insulin release *in vitro*. The expression of prepro-TRH mRNA was only partially reduced in the islets of adult nSTZ rats when compared to controls. nSTZ rats had normal levels of plasma glucose and insulin but the islets of nSTZ rats failed to response by increased insulin secretion to stimulation with 16.7 mmol/l glucose or 50 mmol/l KCl. Perinatal TRH treatment enhanced basal insulin secretion *in vitro* in nSTZ animals of both sexes and partially restored the insulin response to glucose stimulation in nSTZ females.

Key words: Streptozotocin — Thyrotropin-releasing hormone — Insulin — Glucose — Islets

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Introduction

Thyrotropin-releasing hormone (TRH), a tripeptide pGlu-His-Pro-NH_2 originally isolated from mammalian hypothalamus (Boler et al. 1969; Burgus et al. 1969), has been also found in insulin-containing B-cells of the islets of Langerhans (Kawano et al. 1983; Leduque et al. 1985). Like many other regulatory peptides, TRH is initially synthesized as a large prohormone, prepro-TRH, which is subsequently processed to TRH and several other non-TRH or TRH-extended peptides (for review see Nillni and Sevarino 1999). The role of pancreatic TRH remains unclear, but there are indications about its possible role in the pancreatic development and involvement of TRH and/or prepro-TRH-derived peptide(s) in the modulation of insulin secretory response to glucose stimulation (Ebiou et al. 1992a; Yamada et al. 1997; Duntas et al. 1998; Benicky and Strbak 2000). The expression of prepro-TRH mRNA culminates in the rat pancreas at the day of birth (Dutour et al. 1987), which closely corresponds with high pancreatic TRH concentration during the first postnatal days (Martino et al. 1980; Dutour et al. 1987). High levels are transient and the expression of the TRH gene and the content of TRH gradually decline after the 4th postnatal day towards the low levels typical for adult animals. The period of high TRH levels coincides with the perinatal maturation of the insulin secretory responsiveness to glucose and other nutrient secretagogues (Grill et al. 1981). The poor secretory response to a glucose challenge in the newborn rat is not due to decreased insulin storage but involves a step in stimulus-secretion coupling (Grill et al. 1981).

Injection of streptozotocin (STZ) to adult rats causes rapid and irreversible destruction of pancreatic B-cells resulting in a severe insulin-dependent diabetic syndrome. When administered neonatally, the acute effect of STZ is followed by spontaneous B-cell regeneration and return to normal glucose levels within about two weeks (Bonner-Weir et al. 1981). Nevertheless, a disturbance of the insulin response to glucose stimulation persists, thus providing a model for type 2 diabetes mellitus (Permutt et al. 1984).

Neonatal STZ (nSTZ) treatment results in parallel depletion of both insulin and TRH in the rat pancreas (Aratan-Spire et al. 1984). It has been reported, that destruction of pancreatic TRH system, in contrast to insulin, is irreversible and does not undergo spontaneous recovery (Aratan-Spire et al. 1984; Leduque et al. 1987). Neonatal pancreas contains different types of insulin-containing cells – insulin is colocalized with glucagon in one subpopulation and with TRH in another one (Basmaciogullari et al. 2000), which particularly may be irreversibly affected with STZ treatment.

Prepro-TRH gene disruption in experimental mice results in hyperglycemia, accompanied by an impaired insulin response to glucose (Yamada et al. 1997). A similar disturbance of insulin secretion is observed in nSTZ animals (Briaud et al. 2000; Hemmings and Spafford 2000). The role of TRH in this defect is therefore of interest. TRH could play a role in insulin regulation during both the neonatal period or/and during the period preceding glucose stimulation. Since TRH does not

seem to have a direct effect on glucose-induced insulin secretion by islets from adult animals (Konturek et al. 1981; and our unpublished observation), in the present study the effect of TRH supplementation during the first week of life on insulin secretion in nSTZ rats was examined.

Materials and Methods

Experimental design

Ten pregnant female Wistar rats (Charles River, Sulzfeld, Germany) were kept under controlled temperature (22–24 ℃) and a constant 12-h light/dark cycle and fed with Purina Chow and tap water ad libitum. On the day of delivery pups of both sexes in each litter (in order to obviate interlitter variability) were divided into three experimental groups as follows:

- Absolute controls – with no treatment.
- Control group (C-) – the animals received a single injection of citrate buffer in the same way as in the STZ group. Since we did not find any difference between data from absolute and citrate controls, the results of both groups were combined (C-group).
- STZ – treated group (STZ-) – on the day of birth the animals received a single injection of STZ (Sigma; 90 mg/kg BW i.p. in 0.1 mol/l citrate buffer; pH 4.6).

The animals of the previous groups were divided into two subgroups receiving:

- No – TRH treatment (-0) – the animals did not receive any TRH treatment (C-0 and STZ-0, intact and STZ-treated groups, respectively).
- TRH treatment (-TRH) – the animals were injected daily with TRH (10 ng TRH/g BW/day s.c.) during the first week of life (C-TRH and STZ-TRH, intact and STZ-treated groups, respectively).

At the age of 12 weeks, blood glucose (from a tail cut) was measured in fed animals under basal conditions in the morning. In the evening tap water was replaced by 10 % glucose solution in the drinking bottles and blood glucose was checked again next morning.

At the age of 12–14 weeks, the animals were anesthetized with Pentobarbital (Spofa, Prague, Czech Republic) and used for separate isolation of pancreatic islets for subsequent *in vitro* studies. The animals had free access to food before anesthesia. Immediately after pancreas excision, the animals were sacrificed by decapitation and their plasma was saved for insulin and glucose determination. The experimental design is summarized in Table 1.

Isolation and incubation of the islets of Langerhans

The islets were isolated from anesthetized rats by collagenase digestion (Lacy and Kostianovsky 1967). Briefly, the hepatic part of common bile duct was cannulated and the distal end was tied near the duodenal exit. The pancreas was then distended by intraductal injection of Hank's Balanced Salt Solution (Sigma, Germany; pH 7.4). The distended pancreas was excised, cut into small pieces and incubated

Table 1. Design of the experiment

Age	Day 0	Days 1–7	Age at testing	Group
Treatment	Vehicle	Vehicle	12–14 weeks	C-0
	Vehicle	TRH	12–14 weeks	C-TRH
	STZ	Vehicle	12–14 weeks	STZ-0
	STZ	TRH	12–14 weeks	STZ-TRH

at 37 ℃ for 12 min with collagenase (type XI, Sigma, MO, USA; 1 mg/ml) in the presence of 0.008 % soybean trypsin inhibitor (Sigma). Liberated islets were isolated by hand-picking using a dissecting microscope, collected in incubation tubes and preincubated in basal medium (see below for composition) for 60 min at 37 ℃. After the preincubation, the islets (30 islets *per* tube in a final volume of 200 μl) were incubated at 37 ℃ in four subsequent 30-min incubation periods interrupted by medium exchange as follows: basal incubation (30 min, basal medium) – 1st stimulation (30 min, 16.7 mmol/l glucose) – basal incubation (30 min, basal medium) – 2nd stimulation (30 min, 50 mmol/l KCl). The incubation medium was saved at the end of each period and used for insulin radioimmunoassay. Krebs–Ringer HEPES buffer (118 mmol/l NaCl, 4 mmol/l KCl, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l KH_2PO_4, 2.5 mmol/l $CaCl_2$, 25 mmol/l $NaHCO_3$, 20 mmol/l HEPES, 3 mmol/l D-glucose, 95 % O_2–5 % CO_2, pH 7.4, 300 mosmol/l) was used as basal incubation medium. The addition of glucose and KCl in stimulatory media was compensated by isosmotic reduction of NaCl concentration to avoid non-specific osmotic influence.

RNA isolation and relative quantification of mRNA levels by RT-PCR

Freshly isolated pancreatic islets from control and nSTZ animals were used for mRNA analysis. RNA was isolated by the procedure of Chomczynski and Sacchi (1987) using GTC and phenol/chloroform extraction. Reverse transcription was performed using Ready-To-Go You-Prime First-Strand Beads^{TM} (Amersham Biosciences) and pd(N)6 primer. PCR specific for prepro-TRH was carried out afterwards using primers TRH1: 5′-GGA CCT TGG TTG CTG TCG ACT CTG GCT TTG-3′ and TRH2: 5′-GCT GGC GTT TTG TGA TCC AGG AGT CTA AGG-3′ yielding a 226 bp fragment. The PCR program included 35 cycles of denaturation at 94 ℃ for 2 min, annealing at 60 ℃ for 2 min and polymerization at 72 ℃ for 1 min. The number of cycles was determined by testing 25, 30, 35, and 40 cycles (not shown), in order to be within the linear range of amplification.

As a control for semiquantitative evaluation of PCR, primers for the housekeeper glyceraldehyde 3-phosphate dehydrogenase (GPH1: 5′-AGA TCC ACA ACG GAT ACA TT-3′; GPH2: 5′-TCC CTC AAG ATT GTC AGC AGC AA-3′) were used to amplify a 309 bp fragment from each first strand sample. After denaturation at 94 ℃ for 5 min, 30 cycles of PCR at 94 ℃, 60 ℃ and 72 ℃ for 1 min each were performed.

PCR products were analyzed on 2% agarose gels and visualized by ethidium bromide. The density of the individual bands was evaluated by Image software.

Assays

Release of insulin *in vitro* was measured by specific radioimmunoassay using monoiodinated insulin (Zorad et al. 1985) and rat insulin (Novo Nordisk) as a standard. The specific rabbit antibody against rat insulin was kindly provided by Dr. Stolba (Institute of Endocrinology, Prague, Czech Republic). Plasma insulin was measured with a rat insulin RIA kit (LINCO Research Inc., St. Charles, MO, USA). Blood glucose (from tail cuts) was measured using glucomer One Touch Basic (Lifescan Inc., CA, USA). Glucose in plasma from decapitated animals was measured by commercial kit Oxochrom glucose Glu 250E (Lachema, Czech Republic).

Statistics

The results are expressed as means ± S.E. (n = 4–12 parallel samples for each experiment). Statistical analysis was carried out by one-way or two-way ANOVA followed by a Tuckey test.

Results

Effect of nSTZ treatment on the expression of prepro-TRH gene in adult rat pancreas

RT-PCR analysis revealed the distinct presence of prepro-TRH mRNA in the pancreatic islets from adult rats. nSTZ treatment slightly decreased ($p < 0.05$) prepro-TRH mRNA levels in the islets of rats of both sexes compared to control animals (Fig. 1). Since the housekeeper GAPDH was expressed almost equally in all samples, we assume that this was not due to decreased viability of the cells.

Body weight (BW)

nSTZ treatment decreased BW of male rats at the age of 21 days (Fig. 2A), but the differences disappeared at the age of 12 weeks (Table 2). Supplementation with TRH reverted BW loss in 21 day-old males back to the values of C-group (Fig. 2A) but had no additional effect on BW in adulthood. No effects of nSTZ or TRH treatment on BW were seen in females up to age of 21 days (Fig. 2B), but supplementation with TRH, in contrast to males, lowered BW of nSTZ females at the age of 12 weeks (Table 2).

Blood glucose and insulin levels

nSTZ treatment did not affect glucose concentration measured in blood from tail cuts in adults. Interestingly, perinatal TRH treatment resulted in higher blood glucose in the nSTZ animals of both sexes ($p < 0.05$, Fig. 3A), although the effect was more profound in the nSTZ males. There were, however, no significant differences among groups after a challenge with overnight drinking of 10% glucose instead of

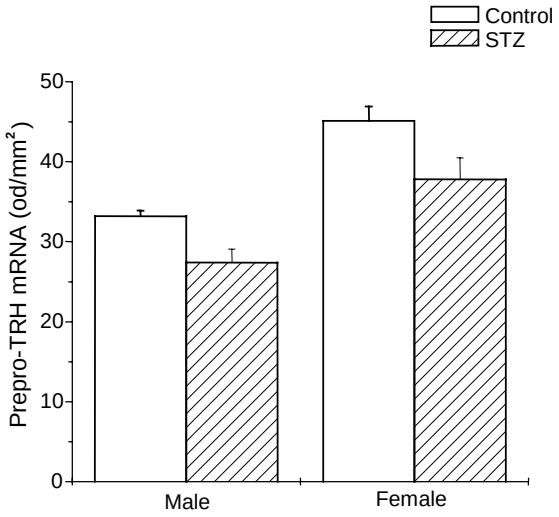

Figure 1. RT-PCR of prepro-TRH mRNA in freshly isolated pancreatic islets from control and nSTZ adult rats. Freshly isolated islets from 12-week-old male and female rats were used for RNA isolation and RT-PCR quantification as described in Methods. Results are expressed as mean ± S.E. ($n = 4$, $p < 0.05$ for both sexes).

Table 2. BW and plasma glucose and insulin levels in nSTZ and TRH-treated rats. Tests were done at the age 12–14 weeks. Glucose and insulin were measured in plasma from decapitated animals following anesthesia and pancreas excision. Results are expressed as mean ± S.E., $n = 4$–12 for each examined group. * $p < 0.05$ for STZ-TRH *vs.* STZ-0

Male				
	C-0	C-TRH	STZ-0	STZ-TRH
BW at the age of 12 weeks (g)	392.9 ± 19.9	395.6 ± 18.6	352.5 ± 13.5	371.7 ± 9.5
Plasma glucose (mmol/l)	9.8 ± 0.3	10.1 ± 1.5	12.3 ± 2.6	14.0 ± 1.7
Plasma insulin (ng/ml)	3.7 ± 0.1	5.1 ± 1.8	1.5 ± 0.1	1.7 ± 0.2
Female				
	C-0	C-TRH	STZ-0	STZ-TRH
BW at the age of 12 weeks (g)	264.7 ± 5.8	250.0 ± 7.4	285.0 ± 10.4	238.6 ± 5.1*
Plasma glucose (mmol/l)	8.5 ± 0.7	9.3 ± 2.2	10.6 ± 1.0	10.6 ± 1.8
Plasma insulin (ng/ml)	4.5 ± 1.5	5.1 ± 2.0	9.9 ± 2.4	5.4 ± 1.0

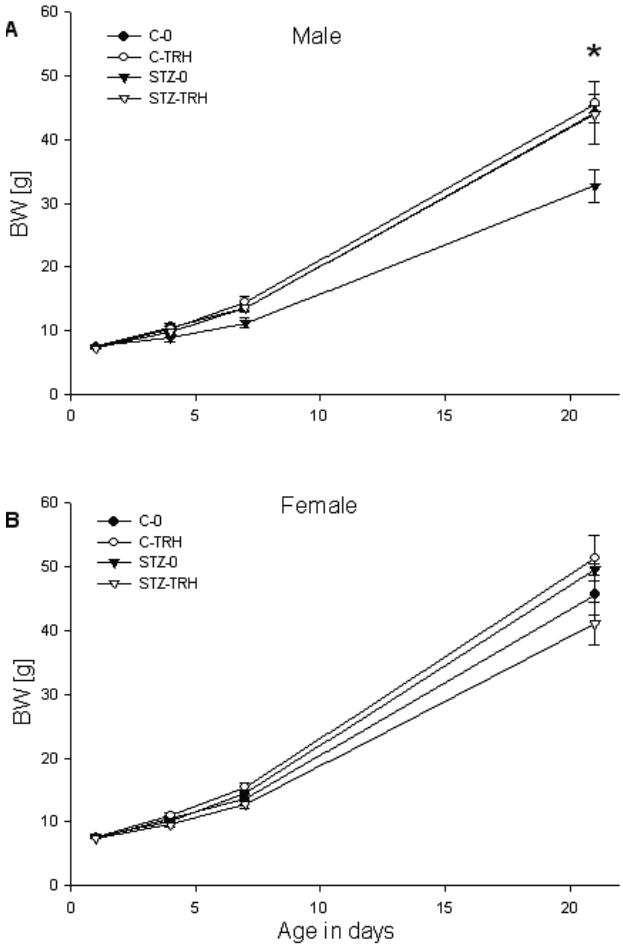

Figure 2. Effect of nSTZ and TRH treatment on BW of male (panel A) and female (panel B) rats. Results are expressed as mean ± S.E.; $n = 4$–12 for each examined group; * $p < 0.05$ for STZ-0 *vs.* C-0 or STZ-TRH, respectively.

tap water (Fig. 3B). When the plasma from blood collected after pancreas excision and decapitation (Table 2) was analyzed, no significant effect of any treatment on plasma glucose or insulin levels between control and treated animals was noted. The plasma glucose levels were, however, higher compared to the levels measured in the blood from tail cuts.

Effect of STZ and TRH treatment on pancreatic islets

During collection under the microscope, smaller size of the islets from nSTZ animals compared to controls was noted.

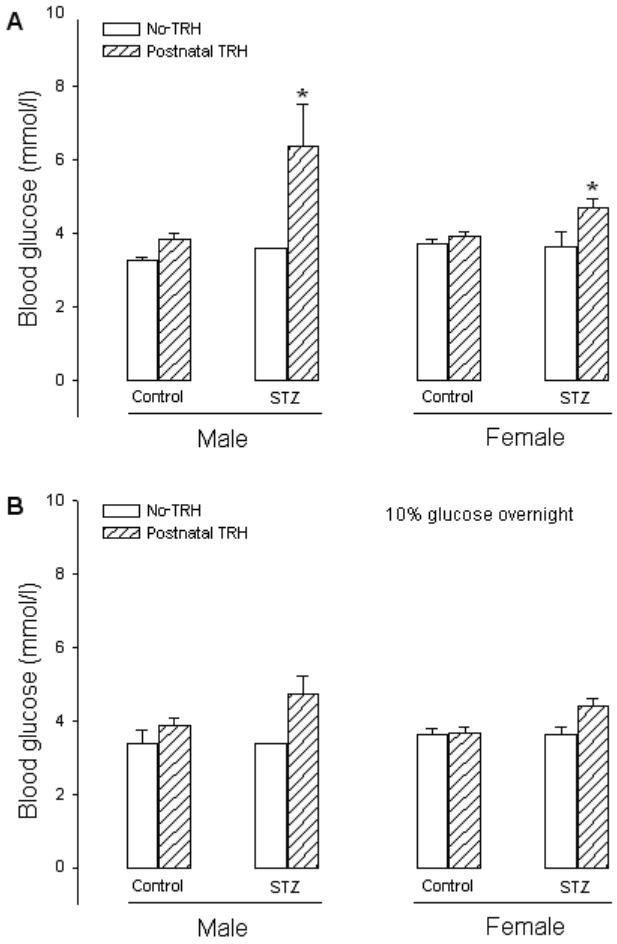

Figure 3. Effect of nSTZ and TRH treatment on blood glucose *in vivo* in animals without (panel A) and with glucose challenge (10 % glucose instead of tap water overnight, panel B). The blood was collected from tail cuts at the age of 12 weeks and measured in fed animals using glucomer One Touch Basic (see Methods for details). Results are expressed as mean ± S.E.; $n = 4$–12 for each examined group; * $p < 0.05$.

Insulin release in response to stimuli

The release of insulin was determined in isolated islets from individual rats incubated alternatively in basal and two different stimulating media (16.7 mmol/l glucose or 50 mmol/l KCl). In islets from control male (Fig. 4A) and female (Fig. 5A) animals, an appropriate increase ($p < 0.001$) of insulin secretion was seen after glucose or depolarizing K^+ stimulation. TRH treatment did not significantly affect the response to both stimuli in these groups of animals. The nSTZ treatment (Fig. 4B

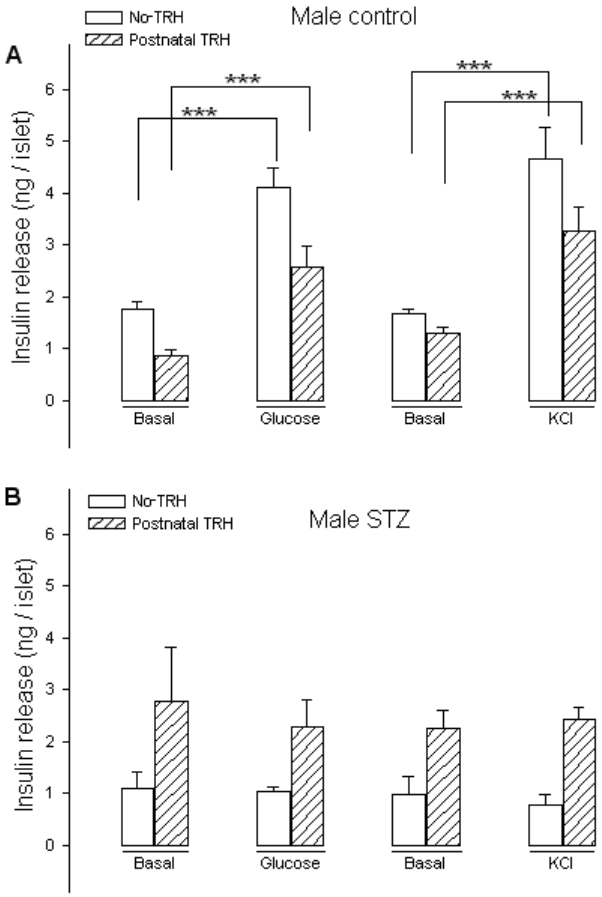

Figure 4. Effect of nSTZ and TRH treatment on *in vitro* insulin release at basal and stimulating conditions by freshly isolated islets of male rats. Panel A: islets (30 *per* tube) of control male rats were preincubated for 60 min at 37 °C and then incubated 4×30 min alternatively in basal and two different stimulating media (16.7 mmol/l glucose and 50 mmol/l KCl, respectively). The medium is indicated below the pairs of columns representing the release of insulin (blank column – vehicle-treated, hatched columns – TRH-treated rats). Results are expressed as mean ± S.E., $n = 4$–2 for each examined group. Panel B: the results for nSTZ male rats. The scheme is the same as in panel A. *** $p < 0.001$.

and Fig. 5B for males and females, respectively) did not affect basal insulin release but the response to both stimuli absented. Perinatal TRH treatment did not correct insulin response to stimulation in nSTZ males but significantly increased the basal insulin secretion (Fig. 4B). In nSTZ females the response to stimulation absented but a response to glucose reappeared ($p < 0.01$) in the TRH-treated nSTZ group (Fig. 5B). The increased secretion lasted until the end of incubation without

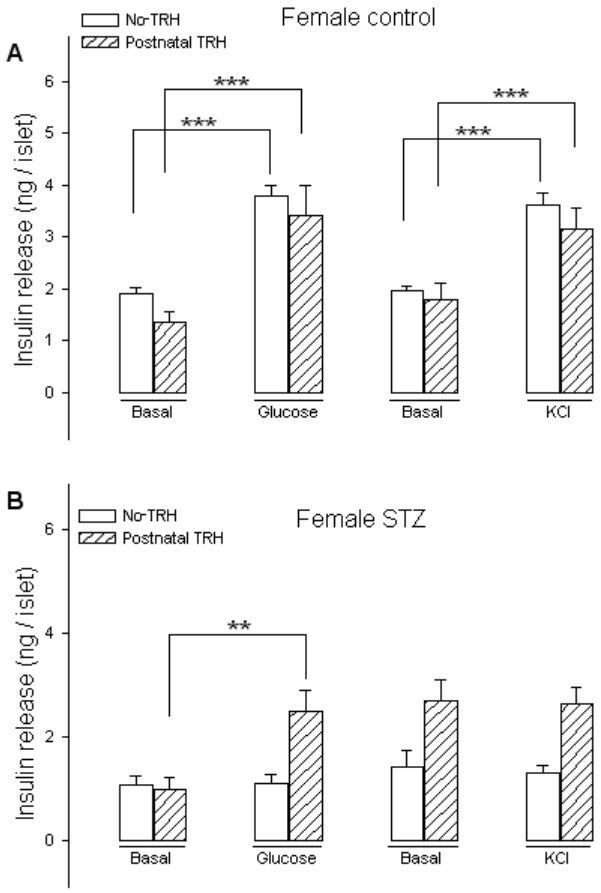

Figure 5. Effect of nSTZ and TRH treatment on *in vitro* insulin release at basal and stimulating conditions by freshly isolated islets of female rats. Panel A: islets (30 *per* tube) of control female rats were preincubated for 60 min at 37°C and then incubated 4×30 min alternatively in basal and two different stimulating media (16.7 mmol/l glucose and 50 mmol/l KCl, respectively). The medium is indicated below the pairs of columns representing the release of insulin (blank column – vehicle-treated, hatched columns – TRH-treated rats). Results are expressed as mean ± S.E., $n = 4$–12 for each examined group. Panel B: the results for nSTZ female rats. The scheme is the same as in panel A. ** $p < 0.01$; *** $p < 0.001$.

a further response to high K^+ depolarizing medium, however. The total amount of secreted insulin (calculated as the sum of release during all four subsequent incubation periods) was lowered in nSTZ animals ($p < 0.001$; Fig. 6) and this decrease was prevented by perinatal TRH treatment both in male ($p < 0.01$) and female nSTZ rats.

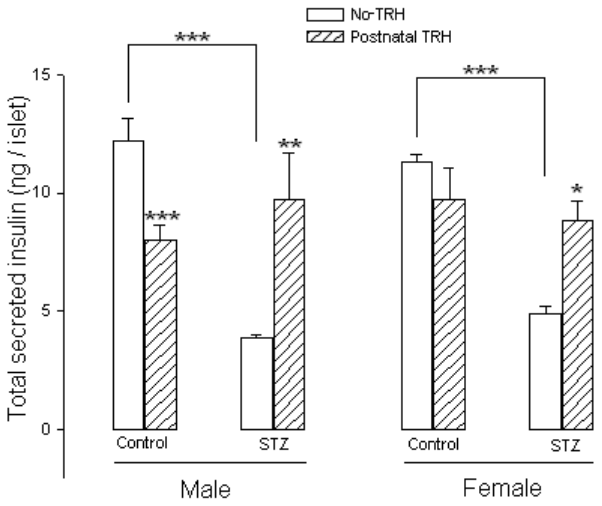

Figure 6. Effect of nSTZ and TRH treatment on the total amount of insulin secreted during incubation. The total amount of insulin released during *in vitro* incubation was calculated as the sum of release of all four consecutive 30 min incubation periods (two basal and two stimulating) as shown on Fig. 4 and Fig. 5, respectively. Results are expressed as mean ± S.E.; $n = 4$–12 for each examined group; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Discussion

Expression of prepro-TRH gene in adult rat pancreas

Our finding of the distinct presence of prepro-TRH mRNA in pancreatic islets from adult rats is at variance with the recent report by Basmaciogullari et al. (2000) that TRH is expressed in the rat pancreas during late embryonic and early postnatal life but they were not able to detect prepro-TRH mRNA in adult rat pancreas. According to the rat TRH mRNA sequence (GI 207467; Lechan et al. 1986) our primers are localized almost in the same position as the primers used by Basmaciogullari et al. (2000). However, as a source of mRNA we have used isolated pancreatic islets instead of whole pancreas as they did. Moreover, we obtained the best results when the annealing temperature was increased to 60 °C and the annealing time was increased to 2 min. Other authors (Fragner et al. 1998) have reported a 4-fold increase of adult islet prepro-TRH mRNA content in hypothyroid rats.

It has been reported that nSTZ treatment, in contrast to insulin, results in irreversible destruction of pancreatic TRH system (Aratan-Spire et al. 1984; Leduque et al. 1987). In contrast with these data, in our study nSTZ treatment resulted only in partial suppression of prepro-TRH mRNA levels in isolated islets of adult animals. This discrepancy can be explained by different approach used for TRH determination. The level of immunoreactive TRH is very low in

adult rat pancreas and is barely detectable by radioimmunoassay used for TRH determination in above-mentioned studies. Moreover, they measured immunoreactive TRH in the extract of whole pancreas homogenates. Pancreatic TRH is present exclusively in B-cells of the islets of Langerhans, which comprise about 2 % of whole pancreatic tissue. We used isolated islets for RT-PCR determination of prepro-TRH mRNA and it showed the distinct presence of TRH expression in adult nSTZ rats. Since gene expression does not necessarily reflect the level of immunoreactive protein, we cannot exclude the possibility, that levels of immunoreactive TRH in nSTZ rats could be posttranscriptionally affected by TRH-degrading activity and/or alternative splicing of TRH-precursor peptide. In fact, it has been reported that nSTZ treatment results in specific enhancement of pancreatic prolyl endopeptidase (a TRH-degrading enzyme) in the neonatal rats, this effect, however, declined with age and disappeared at the age of 20 days (Salers 1994).

Body weight (BW)

STZ- treated males tended to lag behind the controls in the BW increment. This tendency was not persistent and disappeared at the age of 12 weeks. This suppressive effect of STZ was not observed in females, which are generally less susceptible to STZ influence (Ostenson and Malsbury 1989). Effect of nSTZ on BW gain in rats has been previously published and our data correspond with that observation. The effect of nSTZ on BW gain was in males reverted by postnatal supplementation with TRH. This effect is surprising because TRH is generally known as anorectic peptide (Konturek et al. 1981; Lin et al. 1983; Miceli and Malsbury 1985) with suppressive effect on food intake *via* its influence on hypothalamic NPY and inhibition of gastric and stimulated exocrine pancreatic secretion (Konturek et al. 1981; Glasbrenner et al. 1990; Gullo 1991; Kemmer et al. 1995; Fragner et al. 1997). The effect of TRH supplementation on growth hormone production offers one possible explanation. It has been shown both *in vivo* and *in vitro*, that TRH induce increase of serum growth hormone during postnatal maturation and this effect disappears during the second week of life due to maturation of the inhibiting hypothalamic control system (Strbak et al. 1981, 1986; Khorram et al. 1983). Increased levels of circulating growth hormone during the first week of life could be therefore responsible for increased BW gain in TRH-treated males. The opposite effect of TRH on BW of nSTZ females, however, does not support this hypothesis. The mechanism of TRH action on the BW gain remains unknown but appears to be dependent on the sex of animals.

Effect of STZ

STZ treatment did not affect significantly the blood and plasma glucose levels in adult rats, in keeping with previously published results (Briaud et al. 2000). There were, however, considerably higher levels of plasma glucose in decapitated animals in comparison to glucose levels measured a day before in blood from tail cuts. Our animals were anesthetized with pentobarbital and underwent pancreas

excision before decapitation. Surgical trauma under pentobarbital anesthesia has been reported to elevate plasma glucose within 15 min as a result of activation of sympathetic pathways (Lautt and Cote 1977). Even pentobarbital itself was able to increase basal glucose and insulin levels in 9–16 week-old rats (Vera et al. 2002). The combination of pentobarbital anesthesia with pancreas excision is therefore the most probable explanation for our observation. *In vitro* testing of the islets showed that nSTZ resulted in both sexes in the impairment of both glucose- (specific stimulus) and high K^+- (plasma membrane depolarizing nonspecific stimulus) induced release of insulin in adulthood. The relatively constant insulin secretion throughout all incubation periods suggests that nSTZ with the dose used had adversely affected transduction of the stimulus to the regulated secretory pathway in B-cells with only basal (constitutive?) insulin secretion persisting.

Effect of TRH

The neonatal pancreas contains different types of insulin-containing cells – insulin is colocalized with glucagon in one population and with TRH in another (Basmaciogullari et al. 2000). STZ treatment of neonatal rats induces a dramatic drop of prepro-TRH mRNA (Dutour et al. 1987) levels in the pancreas when checked 24 h later and a parallel depletion of insulin and TRH (Aratan–Spire et al. 1984; Leduque et al. 1987). Although the acute effect of STZ is followed by spontaneous B-cell regeneration and normalization of glycemia within two weeks (Bonner–Weir et al. 1981), a disturbance of the insulin response to glucose stimulation persists for the rest of life (Permutt et al. 1984). The similar disturbance was observed in mice with prepro-TRH gene disruption (Yamada et al. 1997). These facts led us to the hypothesis, that the absence of islet TRH could play a role in the disturbance of insulin responsiveness in nSTZ rats. TRH is stored in the same cells and at least during the neonatal period even in the same secretory granules as insulin (Leduque et al. 1989). Moreover, expression of native TRH receptor gene was shown in mouse pancreatic islets and a hamster beta cell line (Yamada et al. 2000) thus raising the possibility of autocrine influence of TRH. In our laboratory we observed a stimulating effect of glucose and an inhibiting effect of insulin on TRH release from adult pancreatic islets (Benicky and Strbak 2000) but we have not seen any effect of TRH on insulin release in short term incubations of islets.

To determine whether there was a specific role of the perinatal absence of pancreatic TRH in the impaired insulin responsiveness in nSTZ animals, we supplemented them with TRH during the first week of life (i.e. the period of the highest pancreatic TRH levels). Perinatal TRH treatment did not significantly affect plasma insulin levels in normal or nSTZ animals in adulthood. nSTZ treatment prevented the response of islets to both glucose and K^+ stimulation in both sexes. In the nSTZ females, an insulin response to glucose reappeared after postnatal TRH supplementation. Recovery was, however, incomplete; the persistence of high secretion thereafter and the absence of a response to the second stimulus suggest impaired function. In contrast to partial recovery in females no effect on glucose-stimulated insulin secretion was seen in STZ-TRH males. This could be attributed

to less damage of islets induced by STZ in females resulting in better reception of corrective manipulations. A poor response to glucose is typical for fetal and neonatal rat pancreas (Grill et al. 1981; Ebiou et al. 1992b) and the fetal character is retained in fetal islet tissue culture (Mourmeaux et al. 1985). The differentiating effect of TRH (Iwasaki et al. 1989; Heritier and Dubois 1993) may play a role in the benefit of neonatally administered peptide and its long-lasting effect in the female rats. However, we do not know if recovery was limited to the response to the first stimulus or specifically to glucose. The possibility, that the dynamics would be similar if the stimuli were used in reverse order cannot be excluded and is supported by the persistence of high insulin secretion in the basal medium in the post-stimulation incubation period, typical for a disturbance of secretion.

Perinatal TRH treatment of STZ rats normalized total production of insulin during incubations of adult islets from both sexes and this correction is due to increased basal, unstimulated (constitutive?) secretion.

Conclusion

nSTZ treatment results in a permanent disturbance of insulin secretion; the defect is more profound in males than in females. Perinatal TRH treatment is followed in adulthood by an increase in basal insulin secretion in nSTZ animals and the appearance of an insulin response to glucose stimulation in nSTZ females.

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