Dual role of the tyrosine kinase GTK and the adaptor protein SHB in β -cell growth: enhanced β -cell replication after 60% pancreatectomy and increased sensitivity to streptozotocin

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Abstract

Transgenic CBA mice expressing either the tyrosine kinase GTK (gut tyrosine kinase) or the adaptor protein SHB (Src homology 2 protein of β -cells) under the control of the rat insulin promoter exhibited an increased β -cell mass, but also elevated cytokine-induced islet cell death compared with control mice. To further investigate the importance of GTK and SHB for β -cell death and proliferation, these mice were subjected to a 60% partial pancreatectomy (Px) or a sham-operation and β -cell replication was determined by autoradiographic detection of [^{3} H]thymidine incorporation into islet cells positively stained for insulin. The Px-operated control mice exhibited a moderate and insignificant increase in β -cell replication 4 days after Px compared with the sham-operated mice ($0.27 \pm 0.08\%$ vs $0.08 \pm 0.02\%$). In contrast, the Px-induced β -cell proliferation was significantly increased in both the GTK- and SHB-transgenic mice compared with the corresponding sham-treated animals ($0.64 \pm 0.12\%$ vs $0.11 \pm 0.04\%$ and $0.44 \pm 0.11\%$ vs

$0.09 \pm 0.04\%$ respectively). This effect was dependent on intracellular signal transduction pathways activated or enhanced by GTK and SHB overexpression, since the proliferation of acinar cells, located in the vicinity of the islets, was equal in the transgenic and control mice. GTK- and SHB-transgenic mice, treated with a sub-diabetogenic dose of the β -cell toxin streptozotocin (STZ) on day 0 and subjected to a glucose tolerance test on day 3, exhibited an impaired glucose tolerance in comparison with the STZ-treated control mice. Pretreatment with STZ blunted the regenerative response to Px in the transgenic mice. Furthermore, the SHB-transgenic islets were significantly more damaged with respect to β -cell loss, compared with the islets of the control mice. Previous and present data suggest a dual role of GTK and SHB for β -cell growth: whereas these proteins increase the β -cell mass and induce β -cell proliferation after 60% Px, SHB and GTK also enhance β -cell death under certain stressful conditions.

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Introduction

Diabetes results from an insufficient β -cell mass or β -cell function to maintain normoglycaemia. The growth and development of the endocrine pancreas has been extensively studied but, despite this, the regulation of the β -cell mass, in both the normal growing pancreas and during the pathogenesis of diabetes remains largely elusive. Regeneration of the exocrine and endocrine pancreas after partial pancreatectomy (Px) has been well documented in animal models and occurs through replication of pre-existing differentiated cells and proliferation of ductal epithelial cells that differentiate to form new pancreatic lobules (Pearson *et al.* 1977, Brockenbrough *et al.* 1988, Bonner-Weir *et al.* 1993). However, the factors inducing pancreas regeneration after Px have not been explored as yet, although various hormones and growth factors have been suggested to be involved in this process.

GTK (gut tyrosine kinase=BSK/IYK), an Src-related tyrosine kinase expressed in islet cells (Öberg-Welsh & Welsh 1995), has recently been shown to be an important regulator of β -cell growth or survival in at least two ways (Annerén & Welsh 2001). Firstly, the β -cell mass of transgenic mice expressing kinase active GTK (Y504F-mutated) under the control of the rat insulin 1 promoter is increased, suggesting a role of GTK in promoting β -cell proliferation or survival. Secondly, islets isolated from transgenic mice show increased cytokine-induced cytotoxicity, suggesting a role of GTK in promoting β -cell death under certain conditions (Annerén & Welsh 2001). GTK has previously been suggested to signal via the Src homology 2-domain adaptor protein SHB (Src homology 2 protein of β -cells) to induce neurite outgrowth in PC12 cells (Annerén *et al.* 2000). Transgenic mice, expressing SHB under the control of the rat insulin 2

promoter, show similar features to the GTK-transgenic mice, with an increased β -cell mass and increased cytokine-induced islet cell death (Welsh *et al.* 1999).

The purpose of the present study was twofold: to determine if GTK or SHB overexpression in β cells influences the regenerative response induced by 60% Px and to investigate the susceptibility of GTK- and SHB-transgenic islets to the β -cell toxin streptozotocin (STZ).

Materials and Methods

Experimental animals

The GTK-transgenic and the SHB-transgenic mice were generated as previously described (Welsh *et al.* 1999, Annerén & Welsh 2001). Approximately 4-month-old GTK-transgenic, SHB-transgenic and control CBA mice of both genders were used for 60% partial Px or sham Px. Male mice (4 months of age) from all groups were used for treatment with STZ. The mice were housed in a temperature-controlled room with a 12 h light:12 h darkness cycle and had continuous access to standard chow and water throughout the study. All procedures were approved by the Animal Ethics Committee in Uppsala, Sweden.

60% pancreatectomy

The mice were anaesthetised with an intraperitoneal injection of 20 ml/kg avertin (a 2.5% (v/v) solution of 10 g 97% 2,2,2-tribromoethanol (Sigma Chemical Co., St Louis, MO, USA) in 10 ml 2-methyl-2-butanol (BDH Chemicals, Poole, Dorset, UK)) and the animals were subjected to 60% Px by removing the entire splenic portion of the pancreas, keeping the duodenal portion intact. Sham-operated mice were handled as above but without removal of the pancreas. The pancreatic remnant is defined as the remaining duodenal portion of the pancreas and the corresponding portion of the sham-treated pancreas is referred to as the remnant equivalent. On days 1, 4 or 14 after the operation the animals were injected with [methyl- ^{3} H]thymidine and the relative β -cell area and labelling index were determined as described below. Fed-state body weight and blood glucose were assessed on day 0, and days 1, 4 or 14 before the mice were killed by cervical dislocation.

Glucose tolerance test

The fed-state glucose tolerance was assessed as follows: mice were injected intraperitoneally or intravenously with 250 μ l 30% (w/v) glucose, and blood glucose was determined (MediSense Pen Sensor; MediSense, Waltham, MA, USA) on venous blood samples collected from the tail

immediately before the glucose injections and after 10, 30, 60 and 120 min.

β -Cell area and labelling index

Mice were injected intraperitoneally with 1 mCi [methyl- ^{3} H]thymidine/kg body weight 1.5 h before cervical dislocation. The pancreas was dissected, fixed in 10% formalin and sectioned. The percentage of insulin-positive area relative to the total pancreas area was determined by staining sections with guinea pig anti-bovine insulin antibody (ICN Biomedicals Inc, Aurora, OH, USA) using an unlabelled peroxidase-antiperoxidase technique (Erlandsen *et al.* 1975). A blinded independent observer determined the insulin-positive area on two cross sections from different regions of each pancreas, using a computerised image analysis system for morphometry (MOP-Videoplan; Kontron Bildanalyse, Munich, Germany).

The labelling index was determined using [^{3} H]thymidine autoradiography on insulin-stained sections by two blinded independent observers (Tyrberg *et al.* 1996). At least 5000 cells were counted from three different regions of each pancreas. Insulin-stained cells were counted to determine the β -cell labelling index whereas acinar cells, in the vicinity of the islets, were counted to determine the acinar labelling index.

Injections of STZ

Four-month-old male mice of all three groups were given a single i.v. injection of STZ (Sigma; 140 mg/kg body weight dissolved in 0.9% NaCl) and the blood glucose was measured on days 0, 3, 7, 14 and 35 after the STZ injection.

Four-month-old male mice were given a single intravenous injection of STZ on day 0 (120 mg/kg body weight). On day 3, the mice were subjected to an intraperitoneal glucose tolerance test as described above. On day 4, the animals were killed or subjected to 60% Px and the β -cell area was determined as described above. β -Cell damage was assessed, using light microscopy by two blinded independent observers on two cross sections from each pancreas as follows: 15–20 islets in each section were ranked from 1 to 5 according to the proportion of insulin-positive cells remaining in the islets. The highest score (5) represents severe β -cell damage with <20% insulin-positive cells. The scores 4, 3 and 2 represent a remaining fraction of β cells in the islets of about 40, 60 and 80% respectively, whereas the lowest score (1) represents a normal-looking islet. Px/STZ-treated mice were injected with [methyl- ^{3} H]thymidine on day 8 and the β -cell labelling index was assessed as described above.

Statistical analysis

Statistics were performed using SigmaStat statistical software (version 2.0 for Windows). The means $\pm$ s.e.m. for

Table 1 Relative β -cell area, body weight, remnant weight and blood glucose after 60% Px. Values are means $\pm$ S.E.M. of number of male (m) and female (f) animals

Group	Number (f/m)	Treatment	β -cell area (%)	Remnant weight (mg)	Whole pancreas weight (mg)	Body weight (day 0/post-Px) (g)	Blood glucose (day 0/post-Px) (mM)
Control	2/2	Sham 4 days	1.4 $\pm$ 0.5	83 $\pm$ 10	209 $\pm$ 10	25 $\pm$ 3/24 $\pm$ 3	7.3 $\pm$ 0.3/7.2 $\pm$ 0.4
Control	5/4	Px 4 days	1.7 $\pm$ 0.4	100 $\pm$ 6	—	26 $\pm$ 1/25 $\pm$ 2	8.6 $\pm$ 0.5/7.2 $\pm$ 0.3
Control	3/4	Px 14 days	1.3 $\pm$ 0.2	141 $\pm$ 9 ^{##}	—	30 $\pm$ 2/28 $\pm$ 2	8.2 $\pm$ 0.3/7.6 $\pm$ 0.3
GTK	2/2	Sham 4 days	2.3 $\pm$ 0.9	94 $\pm$ 10	245 $\pm$ 12*	27 $\pm$ 3/27 $\pm$ 3	8.3 $\pm$ 0.3/7.4 $\pm$ 0.9
GTK	5/4	Px 4 days	2.6 $\pm$ 0.4	108 $\pm$ 11	—	27 $\pm$ 2/26 $\pm$ 1	8.3 $\pm$ 0.6/7.2 $\pm$ 0.6
GTK	3/3	Px 14 days	2.4 $\pm$ 0.3	156 $\pm$ 9 ^{##}	—	31 $\pm$ 2/29 $\pm$ 2	9.1 $\pm$ 0.5/9.1 $\pm$ 1.1
SHB	2/2	Sham 4 days	1.9 $\pm$ 0.5	86 $\pm$ 9	208 $\pm$ 9	24 $\pm$ 4/24 $\pm$ 3	8.1 $\pm$ 0.2/7.0 $\pm$ 0.7
SHB	5/4	Px 4 days	2.1 $\pm$ 0.3	91 $\pm$ 7	—	25 $\pm$ 2/24 $\pm$ 4	7.6 $\pm$ 0.4/7.4 $\pm$ 0.5
SHB	3/4	Px 14 days	1.7 $\pm$ 0.5	120 $\pm$ 9 [#]	—	27 $\pm$ 2/25 $\pm$ 2	7.1 $\pm$ 0.5/7.2 $\pm$ 0.7

Animals in this table are those used for glucose tolerance test and labelling index presented in Figs 1 and 2. Remnant weight is either weight of remaining pancreatic tissue in Px animals or the remnant equivalent from sham-treated animals.

[#] $P < 0.05$, ^{##} $P < 0.01$ compared with corresponding sham value; * $P < 0.05$ compared with corresponding value of control mice.

the number of observations are given. Each observation is based on the value obtained from one animal. One-way analysis of variance followed by a multiple pair-wise comparison procedure using the Student–Newman–Keuls method was used to determine the statistical differences between groups. Spearman Rank Order Correlation was used to measure the association between two variables.

Results

Increased β -cell proliferation 4 days after 60% Px in the GTK-transgenic mice

The non-fasting blood glucose at baseline and 4 days after surgery was equal in all groups. A 60% Px did not increase the blood glucose values (Table 1) or impair the glucose tolerance in any of the experimental groups (Fig. 1), consistent with data obtained from 60% Px rats (Liu *et al.* 2000). The weight of the whole pancreas was significantly larger in the sham-operated GTK-transgenic group compared with the control group, in line with previous results (Annerén & Welsh 2001). The weight of the Px remnant was significantly higher than that of the sham-remnant equivalent after 14 days and was around 60% of the total pancreatic weight in all three groups, whereas the sham-remnant equivalent was around 40% in all groups (Table 1). The relative β -cell area was significantly larger ($P < 0.01$) in the GTK-transgenic animals, as assessed by comparing the pooled data from all GTK groups with the pooled data from the control groups (2.5 ± 0.2 vs 1.5 ± 0.2), consistent with previous observations (Annerén & Welsh 2001) (Table 1).

The β -cell replication, both when described as β -cell labelling index (Fig. 2A) and when related to the acinar labelling index (Fig. 2C), was significantly increased 4 days post-Px in the GTK-transgenic mice compared with the

corresponding sham-operated mice and Px-operated control mice. SHB-transgenic mice also displayed a significant increase in thymidine incorporation in response to Px, whereas the increase in the control mice was smaller and failed to reach significance. There were no detected alterations in glucose tolerance or β -cell proliferation 1 day after surgery (results not shown), consistent with reports from Px rats (Pearson *et al.* 1977). The observed proliferative effects, induced by GTK and SHB, were lost 14 days post-Px, suggesting a transient stimulation of β -cell replication in response to Px by these proteins (Fig. 2). There

Figure 1 Glucose tolerance after 60% Px or sham treatment. GTK-transgenic, SHB-transgenic and control CBA mice underwent an intravenous glucose tolerance test 4 days after 60% or sham Px and blood glucose was measured at the indicated time-points. Data are means $\pm$ S.E.M. from 4–9 mice in each group.

were no apparent differences between the groups in the proliferation of the exocrine, acinar tissue located in the vicinity of the islets, indicating that the observed differences in thymidine incorporation is β -cell specific and thus dependent on expression of the transgenes (Fig. 2B).

Increased sensitivity to STZ

A single high dose of 160–200 mg/kg STZ is usually required to induce β -cell destruction and hyperglycaemia in mice. To test the susceptibility of *GTK*- and *SHB*-expressing β cells to STZ, a sub-diabetogenic dose of 140 mg/kg was injected into transgenic and control mice. Only male mice were used for this experiment because of the reported sex differences in the hyperglycaemic response to multiple low doses of STZ (Rossini *et al.* 1978, Welsh *et al.* 1999). The non-fasting blood glucose on day 0 was similar in all groups but increased with time in response to STZ. The rise in blood glucose was, however, more prompt and exacerbated in the *SHB*- and *GTK*-transgenic mice compared with the control mice (Fig. 3A), with significantly higher glucose levels in the *SHB*-transgenic animals 3 days after the STZ injection. When mice were injected with an even lower dose of STZ (120 mg/kg) and subjected to an intraperitoneal glucose tolerance test on day 3, the STZ treatment again significantly increased blood glucose levels in the *SHB*-transgenic mice compared with the control mice. In the control mice, the glucose challenge induced a rise in blood glucose to above 15 mM at 30 min; this diminished rapidly and returned to near-normal levels after 120 min. The *GTK*-transgenic and *SHB*-transgenic mice, however, displayed a severely perturbed glucose tolerance with significantly elevated blood glucose at all time-points (Fig. 3B).

To investigate whether the impaired glucose tolerance was correlated with an increased β -cell damage, we scored insulin-stained sections from 1 to 5 with respect to β -cell loss (a higher score represents more damaged islets). The *SHB*-transgenic group showed significantly increased β -cell damage with an average score of nearly 3, whereas there were no significant differences between the *GTK*-transgenic and control group (Table 2). The individual scores for the *SHB*-transgenic mice correlated well with the basal blood glucose on day 4 and six out of the seven highest ranked animals were all hyperglycaemic and belonged to the *SHB* group (Fig. 4D). There was no correlation between the scoring and the basal blood glucose in the *GTK*-transgenic group, with certain

Figure 2 β -Cell labelling index after 60% Px or sham treatment. *GTK*-transgenic, *SHB*-transgenic and control CBA mice were given an intraperitoneal injection of [^{3} H]thymidine 4 or 14 days after 60% ($n=4-9$) or sham ($n=4$) Px. The pancreas was fixed, sectioned and subjected to insulin staining and autoradiography. (A) The β -cell labelling index as percent insulin-positive cells with nuclei that have incorporated [^{3} H]thymidine. (B) The acinar cell-labelling index as percent acinar cells, located in the vicinity of the islets with nuclei that have incorporated [^{3} H]thymidine. (C) The relative β -cell labelling index calculated as β -cell labelling index divided by acinar cell labelling index for each animal. Data are means $\pm$ S.E.M. $\#P<0.05$, $\#\#P<0.01$ when compared with the corresponding value of the sham-operated mice; $*P<0.05$ when compared with the corresponding value in the CBA control mice.

Figure 3 Blood glucose profile after a single intravenous injection of STZ. (A) Male mice (4 months old) were injected with STZ (140 mg/kg body weight) and the blood glucose levels were determined immediately before the injection and on days 3, 7, 14 and 35. Data are means $\pm$ S.E.M. for 6–10 mice. ** $P < 0.01$ when compared with the corresponding value in the CBA control mice. (B) Male mice (4 months old) were injected with STZ (120 mg/kg body weight) and the blood glucose was determined on day 4 before and at 30, 60, 120 min after a glucose challenge of 250 μ l 30% glucose, injected intraperitoneally. Data are means $\pm$ S.E.M. for 15–16 mice. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ when compared with corresponding value in the CBA control mice.

animals receiving a high rank despite a low basal glucose level (Fig. 4B). This could be partly explained by the increased β -cell mass of these animals, which might act as a reservoir that prevents hyperglycaemia despite excessive β -cell loss. Indeed, the two animals with the most damaged islets exhibited a total β -cell area which was above 1.9% of the total pancreas.

Table 2 Morphological evaluation of β -cell damage in insulin-stained pancreas sections after treatment with STZ. Mean score $\pm$ S.E.M. is calculated from 14–16 mice

	<i>n</i>	1	2	3	4	5	Mean score
Group							
Control	16	6	5	5	—	—	1.9 $\pm$ 0.2
GTK	15	1	9	4	1	—	2.3 $\pm$ 0.1
SHB	14	—	4	6	4	—	3.1 $\pm$ 0.2**

The values are number of animals ranked within each interval (i.e. 1=1–1.4, 2=1.5–2.4, 3=2.5–3.4, 4=3.5–4.4 and 5=4.5–5). The animals used in this table are those presented in Fig. 4.

** $P < 0.01$ when compared with the corresponding value of the control.

STZ impairs the ability of GTK and SHB to stimulate Px-induced β -cell replication

To determine if STZ affects the ability of GTK and SHB to stimulate β -cell replication 4 days post-Px, mice were injected with STZ (120 mg/kg) on day 0 and subjected to a 60% Px or sham operation on day 4. In contrast to the non-diabetic mice (as shown in Fig. 2), 60% Px did not stimulate β -cell replication on day 4 in any of the STZ-treated groups (Fig. 5). There was a small but insignificant increase in thymidine incorporation in the sham-operated transgenic mice compared with the corresponding controls ($P = 0.25$); this might be explained by the STZ-induced hyperglycaemia or impaired glucose tolerance in these mice, since glucose has been shown to stimulate β -cell proliferation (Bonner-Weir *et al.* 1989). The relative β -cell area of the STZ-treated control mice was similar to the β -cell area of the untreated control mice (1.7 ± 0.2 vs 1.4 ± 0.5). In contrast, the relative β -cell area of the STZ-treated SHB and GTK mice appeared to be reduced compared with the untreated mice (1.2 ± 0.3 vs 1.9 ± 0.5 and 1.7 ± 0.3 vs 2.3 ± 0.2 respectively) and was similar to that of the control mice (Table 3). This reinforces the view that STZ induces a severe β -cell loss in the SHB- and GTK-transgenic mice which is not apparent in the control mice under these conditions of sub-optimal STZ treatment.

Discussion

Type 1 diabetes results from a selective destruction of β cells and a limited capacity of the remaining β cells to regenerate in a compensatory manner. Thus, factors that can upregulate or initiate β -cell proliferation might be of great importance in tilting the balance towards regeneration and perhaps avoiding the manifestation of diabetes. The normal adult rodent pancreatic islet cells are to a large extent terminally differentiated but retain the capacity to regenerate 2–3% of the β cells per day (Svenne 1983). In addition to this low basal level of β -cell proliferation, it has been shown that the pancreas senses a deficit in its mass

Figure 4 Basal blood glucose versus β -cell damage 4 days after STZ treatment. Male mice (4 months old) were injected with STZ (120 mg/kg body weight) and the blood glucose was determined on day 4 before the mice were killed and the pancreas dissected. Formalin-fixed sections were subjected to insulin immunohistochemistry and the islets were ranked 1–5 by two blinded observers according to the β -cell loss. The highest score represents severe damage with less than 20% insulin-positive cells remaining in the islet. The basal blood glucose (mM) was plotted against the average score, calculated from at least 15 islets from each individual mouse: (A) CBA control mice ($n=16$) $y=4.7343+2.8406x$ ($r=0.372$; $P=0.153$); (B) GTK-transgenic mice ($n=15$) $y=9.8211-6.6018e-2x$ ($r=-0.064$; $P=0.812$); (C) SHB-transgenic mice ($n=14$) $y=6.3226+3.4884x$ ($r=0.617$; $P=0.018$); (D) pooled data from all groups. * $P<0.05$ when basal blood glucose was correlated against score, using Spearman Rank Order Correlation.

upon Px and regenerates in order to restore it (Pearson *et al.* 1977, Brockenbrough *et al.* 1988, Bonner-Weir *et al.* 1993, Hardikar *et al.* 1999).

In the present study, the capacity for β -cell replication in GTK- and SHB-transgenic mice was determined after surgical resection of about 60% of the pancreas. Px neither affected the basal glucose levels nor the glucose tolerance in any of the groups, consistent with reports from 60% Px

rats, showing insulinaemia, glycaemia and triglyceridaemia indistinguishable from that of sham-operated rats (Liu *et al.* 2000). The extent of pancreas regeneration is positively correlated with the degree of resection (Pearson *et al.* 1977) and this might be explained by the hyperglycaemia that occurs after large resections (Bonner-Weir *et al.* 1983), since glucose has been shown to stimulate β -cell proliferation (Bonner-Weir *et al.* 1989). Therefore,

preserved normoglycaemia is plausible explanation for the failure of the control mice to significantly stimulate β -cell replication upon Px.

The increased β -cell mass (Annerén & Welsh 2001) and the transiently increased proliferation, in response to Px, in

Table 3 Relative β -cell area after STZ treatment and 60% Px. Values are means $\pm$ S.E.M.; *n* is the number of animals in each group (4-month-old male mice)

	Treatment	<i>n</i>	β -cell area (%)
Group	Sham+STZ	7	1.7 $\pm$ 0.2
	Px+STZ	7	1.4 $\pm$ 0.5
GTK	Sham+STZ	8	1.7 $\pm$ 0.3
	Px+STZ	7	1.7 $\pm$ 0.4
SHB	Sham+STZ	8	1.2 $\pm$ 0.3
	Px+STZ	5	1.2 $\pm$ 0.3

Animals are those used for labelling index in Fig. 5.

GTK-transgenic mice suggest a role for GTK in the stimulation of β -cell growth. It is presently not clear how GTK could bring this about but the increased proliferative effect was limited to the GTK-overexpressing β cells, suggesting that GTK exerts intracellular effects by activating one or more mitogenic pathways. It is unlikely that GTK overexpression induces secretion of a hormone or trophic factor, since this would probably result in increased proliferation of non-insulin-positive cells in the vicinity of the islets. Previous studies have shown an increased basal extracellular signal-regulated kinase (ERK) 1/2 activity in isolated GTK-transgenic islets (Annerén & Welsh 2001) and this might have consequences for proliferation since it is known that ERK1/2 promotes cell growth in many cell types. Moreover, the mRNA levels of proteins upstream of ERK, such as RAS and RAF, have been shown to be elevated in response to Px (Silverman *et al.* 1990, Calvo *et al.* 1991), suggesting a role for this pathway in Px-induced regeneration. SHB overexpression also increases the relative β -cell area and β -cell proliferation in response to 60% Px, in a manner similar to that observed for GTK (Annerén & Welsh 2001). GTK has been suggested to phosphorylate and signal via SHB in GTK-expressing PC12 cells (Annerén *et al.* 2000, Lu *et al.* 2000), and it is therefore possible that SHB is involved in GTK signalling in insulin-producing cells as well. Consistent with this hypothesis is that GTK and SHB exert similar responses in transgenic animals and insulinoma cell lines overexpressing these proteins. For instance, apart from both proteins increasing the β -cell mass, both enhance cytokine-induced cytotoxicity in insulin-producing cells (Welsh

Figure 5 β -Cell labelling index after STZ treatment and 60% or sham Px. The β -cell labelling index, as percent insulin-positive cells with nuclei that have incorporated [^{3} H]thymidine. GTK-transgenic, SHB-transgenic and control CBA male mice (4 months old) were injected with STZ (120 mg/kg) on day 0. On day 4 the mice were subjected to 60% (*n*=6) or sham (*n*=6) Px. On day 8 they were given an intraperitoneal injection of [^{3} H]thymidine and the pancreata were fixed, sectioned and subjected to autoradiography and insulin staining.

et al. 1999, Annerén & Welsh 2000, 2001). Taken together, present and previous results suggest the following hypothesised model: an operative insult and/or deficit in pancreas mass somehow triggers the expression of trophic factors. One or more of these factors signal via GTK and/or SHB, which might stimulate β -cell proliferation by activating the RAS/ERK pathway.

The present study also demonstrated an increased *in vivo* susceptibility to the β -cell-specific toxic substance STZ. The toxin not only increased blood glucose and impaired glucose tolerance in the GTK- and SHB-transgenic mice, but also induced a more prominent β -cell loss. Moreover, prior STZ treatment almost completely abolished the GTK- and SHB-dependent β -cell replication after 60% Px, suggesting that STZ caused severe damage to the GTK- and SHB-overexpressing β cells. This is partly in line with a previous study showing higher blood glucose values of SHB-transgenic mice compared with control mice after multiple low doses of STZ, and with studies showing increased cytokine-induced toxicity of SHB- and GTK-transgenic islet cells (Welsh et al. 1999, Annerén & Welsh 2001). STZ and cytotoxic cytokines induce DNA damage leading to poly-ADP ribose polymerase activation, depletion of NAD⁺ and subsequent cell death (Yamamoto et al. 1981a,b, Fernandez-Alvarez et al. 1994) or impaired β -cell function. In contrast to cytokines, however, STZ has been shown to induce permanent β -cell dysfunction and the remaining population of partially damaged β cells fail to maintain adequate insulin biosynthesis and release (Strandell et al. 1988, Eizirik et al. 1993, Inoue et al. 1994). Based on this, the STZ-induced DNA damage might be similar in the transgenic and control mice but, whereas the population of partially damaged β cells survive in the control mice, SHB and GTK aggravate the response to DNA damage by inducing, yet unidentified, pathways resulting in cell death or chronic dysfunction. One such pathway might involve the activation of ERK1/2, since this kinase has been suggested to play a role in cytokine-induced cell death (Pavlovic et al. 2000) and since GTK increases ERK activity in transgenic islets and GTK-expressing RINm5F cells (Annerén & Welsh 2000).

In summary, the present and previous studies demonstrate a dual role of SHB and GTK in β -cell growth: whereas GTK and SHB both increase the relative β -cell mass (Welsh et al. 1999, Annerén & Welsh 2001) and enhance β -cell proliferation after 60% partial Px, they also increase β -cell death under certain stressful conditions (Welsh et al. 1999, Annerén & Welsh 2000, 2001), suggesting a possible role of SHB and GTK for β -cell destruction in type 1 diabetes.

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