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Transgenic mice overexpressing α_{2A}-adrenoceptors in pancreatic beta-cells show altered regulation of glucose homeostasis

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Abstract

Aims/hypothesis. To study the role of the human α_{2A}-adrenoceptor in the regulation of insulin secretion and the maintenance of glucose homeostasis in transgenic mice overexpressing this receptor in pancreatic beta cells.
Methods. A human insulin promoter/human α_2C10-adrenoceptor chimeric gene was microinjected into mouse embryos and transgenic mice were obtained.
Results. Analysis by RT-PCR showed that the expression of the transgene was restricted to pancreatic islets. Study of the binding of the α_2-antagonist [^{3}H]RX821 002 to membrane preparations showed that islets from transgenic mice had ninefold higher α_2-adrenoceptor density than those from controls. Immunohistological analysis showed, however, no change in the number or size of islets between control and transgenic mice. Transgenic animals had normal glycaemia and insulinaemia in basal conditions but greater hyperglycaemic and hypoinsulinaemic responses after injection of the α_2-agonist, UK14 304. The lower blood insulin concentration detected in transgenic mice was a reflection of a stronger inhibitory effect of the α_2-agonist on glucose-stimulated insulin secretion in transgenic islets than in controls. Furthermore, transgenic mice did not have lower glycaemia to basal values after an intraperitoneal glucose tolerance test. This defect was abolished by treatment with the α_2-adrenoceptor antagonist, RX821 002.
Conclusion/interpretation. These results provide evidence in vivo that overexpression of α_2-adrenoceptors in beta cells can lead to impaired insulin secretion and glucose intolerance. [Diabetologia (2000) 43: 899–906]

Keywords Diabetes, insulin, catecholamines, α_2-agonist, adrenoceptor, islets, transgenic mice.

The regulation of insulin release from pancreatic beta cells is a highly complex process, integrating glycaemia value as well as the modulatory actions of many hormones, neuropeptides and neurotransmitters [1]. The endocrine pancreas is richly innervated by sympathetic neurons and electrical stimulation of splanchnic nerves or treatment with epinephrine results in strong inhibition of insulin secretion [2]. Pharmacological studies carried out both in vivo [3–4] and on isolated islets [5] show that this inhibitory effect is primarily due to stimulation of peripheral adrenoceptors of the α_2-type. Consistent with this, binding studies with selective radioligands showed the presence of α_2-adrenoceptors on membranes from pancreatic islets isolated from various species [6] as well as on membranes from a rodent insulinoma [7]. The α_2-adrenoceptor family consists of three receptor subtypes (α_{2A}, α_{2B} and α_{2C}) encoded by distinct genes that do not have introns [8]. Transcripts encoding α_{2A}- and α_{2B}-subtypes were detected recently in human [9] and rat [10] pancreatic islets. On the basis of functional studies, the α_{2A}-subtype is, however, responsible for inhibition of insulin secretion [11–12].

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Abbreviations: α_2*C10AR*, Human α_{2A}-adrenoceptor gene; RX821002, 2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline; UK14304, 5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline.

It is thus generally accepted that the hyperglycaemic response to α_2-agonists is mainly due to the inhibition of insulin secretion through stimulation of postsynaptic α_{2A}-adrenoceptors located on beta cells.

It has also been suggested that catecholamines and α_2-adrenoceptors are involved in the aetiology and pathogenesis of Type II (non-insulin-dependent) diabetes mellitus, which is characterised by an impaired insulin response to glucose challenge. The plasma concentrations of catecholamines are higher in diabetic than in healthy subjects [13–14] and glucose intolerance is frequently observed in patients with pheochromocytoma [15–16]. Furthermore, islets isolated from rats that had received neonatal streptozotocin injections (a model of Type II diabetes) were more sensitive to α_2-adrenergic inhibition of glucose-induced insulin release than islets from controls [17–18]. Hence it has been postulated that, in some diabetic patients, insulin secretion is pathologically restrained because of increased tonic inhibition by α_2-adrenoceptors. Subsequently, α_2-blockers were viewed as potentially useful adjuncts in the management of Type II diabetes and compounds such as phentolamine, MK912 and SL 84.0418 were shown to be efficient hypoglycaemic agents [19–21]. The precise physiological role of the beta-cell α_2-adrenoceptors in the regulation of insulin secretion is, however, still a matter of discussion. Pharmacologic data in isolated islets are difficult to interpret because there is a possibility that in islets (nor)adrenaline or alpha-agonists influence presynaptic intraislet nerve endings [22]. Moreover, noradrenaline not only affects insulin release but also glucagon and somatostatin secretion, creating a complex paracrine situation. Furthermore, beta-cell lines might not be a good alternative for whole islets because among the several possible different alpha 2-receptor subtypes that have been cloned, an expression pattern for normal beta cells distinct from that of beta tumour cells has been described [23]. Nevertheless, the study of α_2-adrenoceptors on purified rat beta cells in vitro has shown that α_2-adrenoceptors are important for glucose-induced insulin release [24]. Results from studies in vivo are also difficult to interpret because of the concomitant effects of α_2-adrenergic compound infusion on other neuroendocrine functions and islet blood supply, which might also affect insulin secretion. For instance, the enhancement of insulin secretion after yohimbine treatment was reduced after vagotomy and abolished by β-antagonists, indicating that activation of cholinergic and noradrenergic systems is involved in the effect of this non-imidazoline α_2-antagonist [25]. The contribution of α_2-adrenoceptor blockade to the restoration of insulin secretion in Type II diabetes is also controversial. The α_2-antagonists of the imidazole family (such as phentolamine, idazoxan and efaroxan) are more potent insulinotropic agents than yohimbine and able to reverse the effect of diazoxide [26]. The greater efficacy of imidazoline-derivatives is the consequence of blockade of K^+ conductance through their direct interaction with the pore-forming subunit Kir6.2 of ATP-sensitive K^+ channels [27].

We examined the role of pancreatic α_{2A}-adrenoceptors in the regulation of insulin secretion by developing transgenic mice overexpressing this receptor subtype in their beta cells. Metabolic studies of this animal model indicate that increased α_2-adrenergic receptivity leads to an exacerbated hypoinsulinaemic response to α_2-agonist and to development of glucose intolerance.

Materials and methods

Construction of the human insulin promoter/human a_2C10 adrenoceptor chimeric gene (hInsp/a_2C10) and generation of transgenic mice. The hInsp/α_2C10 transgene consists of a 2 kb fragment of human insulin promoter fused to the coding region of the human α_{2A}-adrenoceptor gene. This construct was obtained as follows: the *Nhe*I-*Hind*III fragment (2 kb) of the α_2*C10* gene containing the entire open reading frame of the human α_{2A}-adrenoceptor [28] was engineered to create *Eco*RI sites at each end. This *Eco*RI/*Eco*RI fragment was then inserted in a new *Eco*RI subcloning site engineered to destroy the ATG translation start site [29] in the *Bam*HI-*Xho*I fragment (4.6 kb) of the human insulin gene [30]. The general procedure for generation of transgenic mice was as described previously [31]. Briefly, the *Bam*HI-*Xho*I fragment (6.6 kb, Fig. 1) containing the entire chimeric gene was purified and microinjected into fertilised eggs obtained from superovulated C57BL6/SJL mice, and viable embryos were reimplanted in the oviducts of pseudopregnant mice. The presence of the transgene was tested by Southern blot analysis. Genomic DNA (10 μg) isolated from tail biopsy specimens of 3-week-old animals were digested with *Eco*RI. Fragments of DNA were separated by agarose gel electrophoresis, blotted and hybridised to a [^{32}P]-labelled cDNA probe prepared from the *Nhe*I-*Hind*III fragment of the α_2*C10* gene. There was no cross-reactivity of this probe with genomic DNA from control mice.

Treatment of animals and determination of hormone and metabolite concentrations. Transgenic mice used were heterozygous for the hInsp/α_2C10 construct. Control and transgenic mice were fed standard mouse chow (Panlab, Barcelona, Spain), given free access to water and kept under a light-dark cycle of 12 h (light on at 0800 hours). The animals were handled according to the "Principle of Laboratory Animal Care" (NIH publication no. 85–23, revised 1985). All experiments were carried out on 2 to 4-month-old animals between 0900 and 1100 hours to minimize circadian variations, α_2-adrenergic drugs were dissolved in saline and given by intraperitoneal injection. Serum insulin concentrations were measured by radioimmunoassay (RIA) (CIS, Biointernational, Gif-Sur-Yvette, France). Serum glucose concentration was measured either enzymatically (Glucoquant, Boehringer Mannheim, Mannheim, Germany) or by using a Glucometer Elite analyser (Bayer, Tarrytown, N.Y., USA). To quantify pancreatic insulin content, whole pancreas was removed from the mice and insulin was extracted by mechanical homogenisation of the organ in 20 vols of cold acid ethanol (75 % ethanol, 1.5 % concentrated HCl) followed by 48 h of agitation at 40 °C. After centrifugation, insulin was quantified in the supernatants of the samples by RIA (CIS, Biointernational).

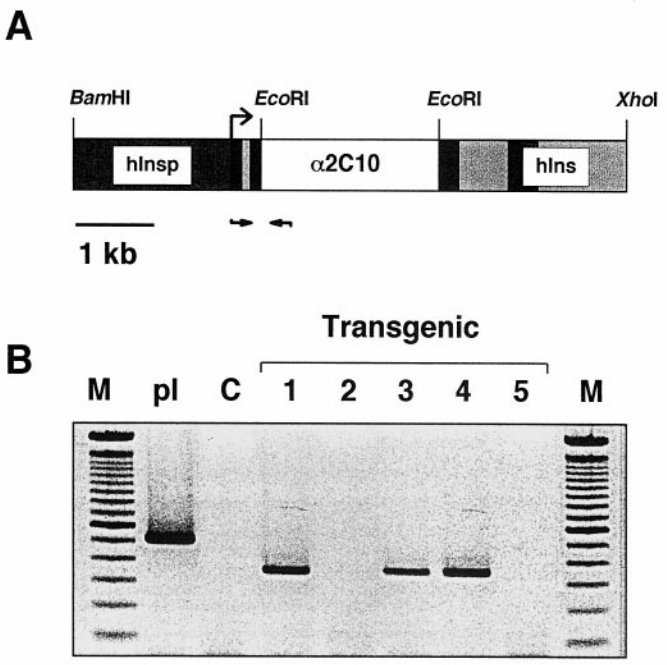

Fig. 1 A, B. Expression of the hInsp/α_2C10 chimeric gene in transgenic mice. **A** Schematic representation of the hInsp/α_2C10 chimeric gene used for obtaining of transgenic mice. The *Nhe*I-*Hind*III fragment (2 kb) of the α_2C10AR gene containing the entire coding region of the α_{2A}-adrenoceptor (white box) was inserted into an *Eco*RI subcloning site created in the human insulin gene as indicated in Methods. The black boxes correspond to the exons of the insulin gene and arrows indicate the position of the sense and antisense primers used in RT-PCR experiments. **B** Analysis by RT-PCR of hInsp/α_2C10 transgene expression in mouse pancreatic islets. Approximately 1 μg of total RNA prepared from isolated islets was reverse transcribed and then subjected to a nested PCR using specific primers as indicated in Methods. Lanes M, 100 bp DNA ladder; lane PL, direct PCR from hInsp/α_2C10 construct ; lane C, RT-PCR from RNA prepared from control mouse and lanes 1 to 5, RT-PCR from RNA prepared from transgenic mice

Glucose and insulin tolerance tests. Control and transgenic mice fasted overnight (16 h) with free access to water were anesthetised with an intraperitoneal injection of 2.5 % avertin solution. They were then given an intraperitoneal injection of either saline or glucose (1 g/kg of body weight). The 2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline (RX821 002) antagonist (10 μmol/kg) was injected 30 min before glucose injection. Blood samples (2 μl) were obtained from tail vein before the glucose injection and at several time points after the glucose load. For the intraperitoneal insulin tolerance test, fed control and transgenic mice were anaesthetised and afterwards injected intraperitoneally with 0.75 IU of a soluble insulin (Humulin regular, Eli Lilly, Indianapols, Ind., USA) per kg body weight. Blood samples were taken from the tail vein of the same animals at several time points and glucose concentration measured.

Immunohistochemical analysis. For immunohistochemical detection of insulin and glucagon, pancreas from control and transgenic mice were fixed for 12 to 24 h in formalin, embedded in paraffin and sectioned. Sections were then incubated overnight at 4 °C with a guinea pig anti-porcine insulin antibody (Dako, Carpinteria, Calif., USA), at 1:100 dilution, or with a rabbit anti-human glucagon antibody (ICN, Costa Mesa, Calif., USA) in 1:4000 dilution. Rabbit anti-guinea pig immunoglobulin G, coupled to peroxidase (Boehringer Mannheim), or biotinylated goat anti-rabbit antibody and ABC complex (Vector, Burlingame, Calif., USA) were used as secondary antibodies. As the substrate chromogen 3'3'-diaminobenzidine (DAB) was used. Sections were counterstained in Mayer's haematoxylin (Merck, Darmstadt, Germany).

Preparation of pancreatic islets. Islets were isolated from the pancreas of control and transgenic mice (2-month-old) fed a standard diet. They were released from pancreatic acinar tissues by digestion with collagenase P (Boehringer Mannheim) and collected by handpicking under a dissection microscope as described previously [32].

RNA analysis. Islet total RNA was extracted by using the guanidine isothiocyanate method [33]. Expression of the transgene was assessed by RT-PCR. First strand cDNA synthesis was done using 1 μg RNA in a 100 μl reaction volume containing ng 1000 U Moloney mouse leukaemia virus reverse transcriptase (Perkin Elmer, Norwalk, Conn., USA) and 0.5 μmol/l oligodT in conditions recommended by the manufacturer. After 1 h at 37 °C the reaction was stopped by 5 min treatment at 95 °C. We amplified cDNA by PCR in a 25 μl reaction volume containing 1 U *Taq* polymerase (Boehringer Mannheim), 0.25 μmol/l of sense and antisense primers, 0.2 mmol/l deoxyribonucleoside triphosphate (dNTP), 10 mmol/l TRIS-HCl, 3 mmol/l $MgCl_2$, 10 % dimethyl sulphoxide (DMSO) and 2.5 % formamide. After 3 min at 94°, the reaction mixture was subjected to 40 cycles of denaturation, hybridation and elongation as follows : 15 s at 940, 30 s at 60°, 1 min at 72°. The sense primer (5′-GGACAGGCTGCATCAGAAGAGG-3′) was located in the first exon of the human insulin gene and the antisense primer (5′-GGCACACCAGCGTCAGCGTCAC-3′) was specific for the α_2*C10* gene. Amplification of the genomic DNA gave a band of 527 bp whereas amplification of the cDNA gave a band of 366 bp.

Receptor quantification. We quantified α_2-adrenoceptors on crude membrane preparations using the α_2-antagonist [^{3}H]RX821 002 as a radioligand [34]. Frozen islets from control and transgenic mice were thawed directly in 5 ml of TE buffer (50 mmol/l TRIS-HCl, pH = 7.5, 5 mmol/l EDTA), disrupted using a Dounce homogeniser, and centrifuged at 25000 × *g* for 10 min. The pellet was washed in TM buffer (50 mmol/l TRIS-HCl, 0.5 mmol/l $MgCl_2$, pH 7.5) in the same conditions as above. The final crude membrane fraction was taken in the appropriate volume of TM buffer for immediate use. The amount of membrane necessary for each binding point corresponded to that obtained from approximately 40 islets. The protein concentration was measured using Bradford's method [35]. Total binding was measured by incubating 100 μl of islet membranes with [^{3}H]RX821 002 (0.5 to 8 nmol/l) in a total volume of 400μl of TM buffer. After a 45-min incubation at 25 °C, bound radioligand was separated from free by passage through GF/C Whatman filters using a cell harvester (Skatron Instruments, Lier, Norway). After washing in ice-cold TM buffer, membrane-bound radioactivity was determined by liquid spectrometry. Specific binding was defined as the difference between total and non-specific binding measured in the presence of 10 μmol/l phentolamine. Data from binding experiments were analysed using the EBDA-ligand computer program [36]

Measurement of insulin secretion from isolated islets. Batches of six islets were incubated in 1 ml of Krebs-Ringer bicarbonate buffer supplemented with 5 mg/ml BSA (Fraction V, Sigma, St. Louis, Mo., USA) and various concentrations of glu-

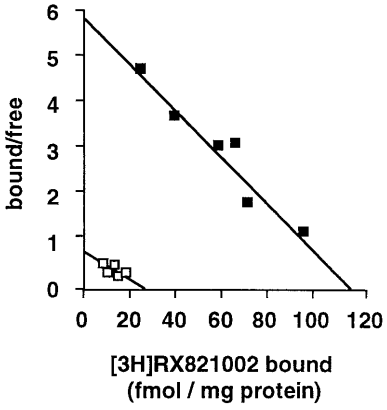

Fig. 2. Scatchard plots of [^{3}H]RX821 002 binding to pancreatic islet membranes. Membranes prepared from pancreatic islets of control (open squares) and transgenic mice (black squares) were incubated in the presence of various concentrations of [^{3}H]RX821 002 (0.1 to 10 nmol.l). The amount of specifically bound radioligand was quantified using 10 μmol/l phentolamine to estimate non-specific binding. The maximum number of [^{3}H]RX821 002 binding sites (B_{max}) were estimated by Scatchard transformation of the saturation isotherms. Three independent experiments indicated that [^{3}H]RX821 002 B_{max} value was respectively 13 ± 4 and 119 ± 17 fmol/mg of protein in control and transgenic islets ($p < 0.005$). No significant change in the equilibrium dissociation constant (K_d) value was observed (1.9 ± 0.4 vs 1.5 ± 0.3 nmol/l)

cose (2.8, 5.5, 11.1 or 16.7 mmol/l). Experiments were carried out in the absence or presence of 10 nmol/l of the α_2-agonist 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK14 304). After incubation for 1 h at 37 °C, tubes were centrifuged and supernatants were stored at –20 °C until the measurement of insulin content by RIA.

Statistical analysis. All values are expressed as the means ± SEM. Statistical analysis was carried out using the Student-Newmann-Keuls test and significance was accepted at the over 95 % confidence level.

Results

Expression of the hInsp/α_2C10AR transgene. Transgenic mice containing an hInsp/α_2C10AR chimeric gene were developed to specifically target overexpression of the α_{2A}-adrenoceptor to beta cells of pancreatic islets. Of the progeny born mouse oocytes were injected, five transgenic founders containing the construct were identified by Southern blot analysis (data not shown). All these founders were successfully bred to establish transgenic lines and RT-PCR analysis was done on RNA isolated from islets. The specific primers chosen for this purpose allowed us to amplify a 366 bp fragment in the endocrine pancreas of three of the transgenic lines (Tg1, Tg3 and Tg4) (Fig. 1). No signal was detected either in control animals or in the other two transgenic lines. Estimation of α_2-adrenoceptor number by measuring radioligand binding was thus restricted to these three transgenic lines expressing the chimeric gene. Analysis of the saturation isotherms of [^{3}H]RX821 002 binding to crude membranes prepared from isolated islets showed that the Tg1 transgenic line had a ninefold higher α_2-adrenoceptor density than control (119 ± 17 vs 13 ± 4 fmol/mg protein) (Fig. 2). No statistically significant increase in the binding capacity was, however, detected in islet membranes from Tg3 or Tg4 transgenic mice compared with controls (data not shown). This indicated that although these transgenic mice expressed the transgene, the concentrations of the protein were probably low. Neither the presence of hInsp/α_2C10AR transcript nor an increase in α_2-adrenoceptor density was observed in the other tissues examined (spleen, brain cortex, kidney), indicating that overexpression of α_2-adrenoceptor was restricted to pancreatic islets. Further studies of the pharmacological properties of the α_2-adrenoceptor in islets of Tg1 transgenic mice showed that yohimbine was 5000-fold more potent than prazosin in inhibiting [^{3}H]RX821 002 binding (data not shown), meaning that the overexpressed receptor was of the α_{2A}-subtype. Immunohistological analysis showed that neither the size and number, nor the respective proportion of insulin-containing and glucagon-containing cells was changed in transgenic islets (data not shown). All subsequent experiments were carried out on 2 to 3-month-old heterozygous Tg1 transgenic mice. Non-transgenic littermates were used as controls.

Serum variables and insulin secretion by isolated islets. No difference in insulinaemia and glycaemia was observed between transgenic and control mice under basal conditions (Fig. 3). A single intraperitoneal injection of the α_2-agonist UK14 304 (1 μmol/kg of body weight) led, however, to an about twofold increase in blood glucose concentration in control animals. As expected for an α_2-agonist, this hyperglycaemic effect correlated with a moderate inhibition of insulinaemia (35 % decrease). The effects of UK14 304 were exacerbated in transgenic mice, which had a 3.5-fold increase in glycaemia and a more pronounced decrease in insulinaemia (76 %).

Studies of the functional response of isolated islets to the α_2-agonist were carried out to determine whether the decrease in serum insulin concentrations correlated with alteration of insulin secretion. Pancreatic islets were thus isolated from fed control and transgenic mice and insulin release was measured at several glucose concentrations in the presence or the absence of 10 nmol/l UK14 304. In the absence of the α_2-agonist, insulin secretion by transgenic islets was identical to that of controls (Fig. 4). At the highest concentration of glucose tested (16.7 mmol/l) an equal induction of insulin secretion (2.5-fold in control vs 2.6-fold in transgenic) was observed, indicating

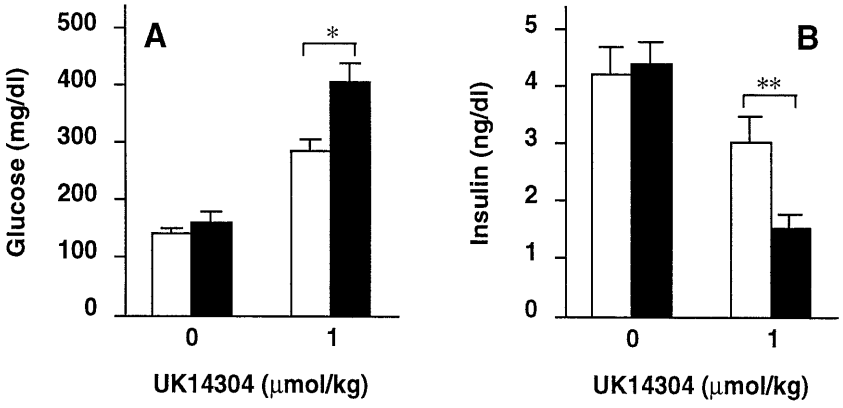

Fig. 3 A, B. Blood glucose and serum insulin concentrations in control and transgenic mice. Blood glucose concentration (**A**) and insulin concentration (**B**) were measured in control (open bars) and transgenic animals (black bars) under basal condition and 30 min after UK14304 treatment (1 μmol/kg). Data represent means ± SEM of 12 to 15 mice per group (* $p < 0.05$, ** $p < 0.02$)

that the secretory response of the islet to glucose was not affected by α_2-adrenoceptor overexpression. Thus, this suggests that signalling induced by glucose is not tonically affected by α_2-adrenoceptors in isolated islets. In contrast, a pronounced difference in the profiles of insulin secretion was detected when UK14304 was added to the incubation medium. The α_2-agonist caused a decrease in insulin secretion whatever the concentration of glucose tested but this effect was significantly greater on islets from transgenic mice exposed to a high glucose concentration (63% decrease in transgenic vs 40% in control at 16.7 mmol/l glucose) (Fig. 4). Measurements of islet glycolysis at three glucose concentrations (2.8, 5.5 and 16.7 mmol/l) in the presence of UK14304 were also done. No difference was found between control and transgenic islets (data not shown), suggesting that the difference in insulin secretion in the presence of α_2-agonist was probably not dependent on further decrease in glucose utilisation. In addition, no difference was noted in pancreatic insulin content between control and transgenic mice (7.1 ± 0.3 μg/100 mg pancreas vs 6.4 ± 0.5 μg/100 mg pancreas), indicating that α_2-adrenoceptor overexpression did not affect beta-cell growth or differentiation.

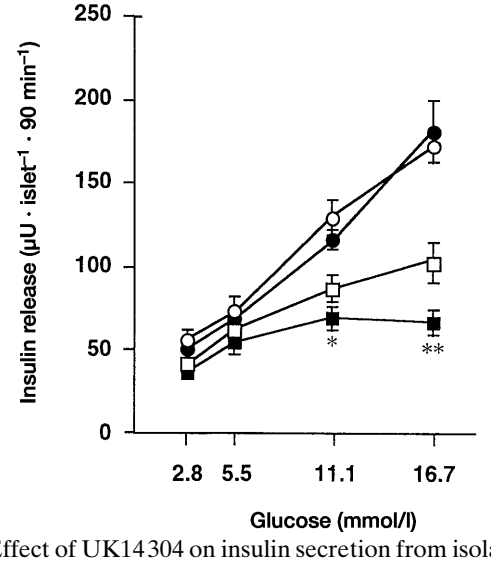

Fig. 4. Effect of UK14304 on insulin secretion from isolated islets. Batches of six islets from control and transgenic mice were incubated for 1 h in Krebs-Ringer bicarbonate buffer containing 2.8, 5.5, 11.1 or 16.7 mmol/l glucose. Insulin secretion was measured in the absence or presence of 10 nmol/l UK14304. Data are presented as means ± SEM of eight individual experiments. * and ** indicate values significantly different from the corresponding in control animals at $p < 0.05$ and $p < 0.02$. ○ control, ● Tg1, □ control + UK 14304, ■ Tg1 + UK 14304

Glucose and insulin tolerance tests. Glucose tolerance tests were carried out to assess whether the differences observed in isolated islets could lead to an alteration of glucose homeostasis after an intraperitoneal glucose load. Glycaemia in starved control animals was nearly the same as in starved transgenics (respectively, 5.7 ± 0.3 and 6.4 ± 0.4 mmol/l). Furthermore, at the different time points after intraperitoneal injection of saline solution both control and transgenic mice had similar blood glucose concentrations (30 min, 6 ± 0.3 vs 6.2 ± 0.2 mmol/l; 60 min, 6.2 ± 0.3 vs 5.9 ± 0.4 mmol/l; 120 min, 6.4 ± 0.4 vs 6.4 ± 0.4 mmol/l). This indicated that handling of animals with the repeated tail snipping in itself did not affect blood glucose concentrations. Intraperitoneal injection of glucose also resulted in a similarily rapid increase of glycaemia (14.2 ± 0.9 mmol/l in control vs 15.3 ± 0.7 mmol/l in transgenic, 30 min after injection) (Fig. 5). Although blood glucose concentration returned to the basal value within 180 min after load in control mice, glycaemia was still high in transgenic mice (12.2 mmol/l). This inability to reduce the concentration of blood glucose suggested that transgenic mice expressing the chimeric gene were glucose intolerant. To establish whether the alteration in glucose tolerance test was exclusively due to α_2-adrenoceptor

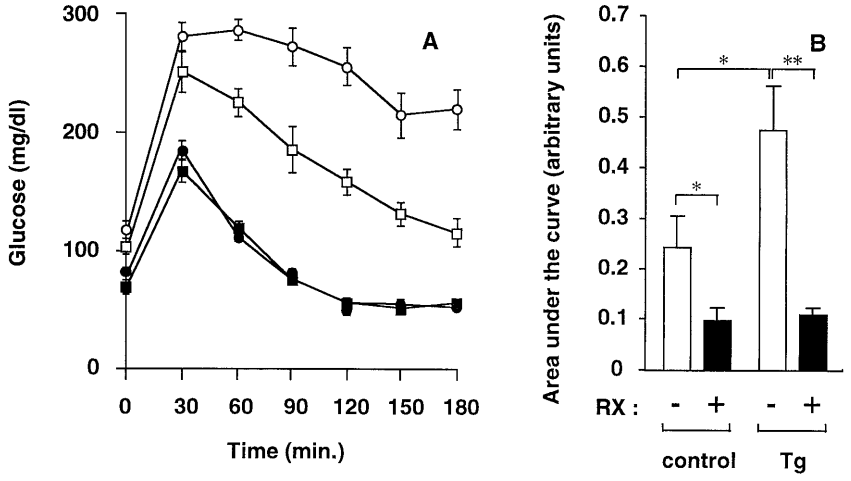

Fig. 5 A, B. Glucose tolerance test. (**A**) Line plots show the blood glucose concentration in control and transgenic mice during glucose tolerance test. Intraperitoneal glucose load (1 g/kg) was given 30 min after injection of saline or RX821 002 (10 μmol/kg). □ control + saline, ○ Tg1 + saline, ■ control + RX, ● Tg1 + RX. (**B**) Bar graphs represent the increase area under the curve in control and transgenic animals having received saline (–) or RX821 002 (+). Data are presented as means ± SEM of six individual experiments (* $p < 0.05$, ** $p < 0.01$).

overexpression, the same experiments were done in the presence of α_2-antagonist RX821 002. Treatment with of RX821 002 before intraperitoneal injection of glucose totally abolished the difference between transgenic and control mice (Fig. 5). Furthermore, restoration of normoglycaemia was faster in mice treated with antagonist than in untreated controls. The differences between the four groups are even more evident when the results are expressed as the increase in area under the curve (Fig. 5). All these findings indicate that overexpression of α_2-adrenoceptors in beta cells induces alterations in glucose tolerance.

When an intraperitoneal insulin tolerance test (injection of 0.75 IU/kg body weight of soluble insulin (Humulin regular, Eli Lilly)) was done in fed mice, a similar hypoglycaemic response was, however, observed in transgenic and in control mice. Thus, by 60 min, both control and transgenic mice showed a 40 % decrease in blood glucose concentrations (Fig. 6), indicating that transgenic mice overexpressing α_2-adrenoceptors in beta cells show altered islet function rather than reduced insulin sensitivity. Therefore, because transgenic mice had normal insulin sensitivity but showed impaired glucose-stimulated insulin secretion in vivo and normal glucose-stimulated insulin secretion in vitro, our study suggests a possible tonic influence of α_2-adrenoceptors in vivo.

Discussion

Several lines of evidence have shown that epinephrine and norepinephrine play a major part in the negative regulation of insulin secretion. The antisecretagogue effect of catecholamines is primarily triggered by the stimulation of α_2-adrenoceptors located on beta cells and it has even been suggested that an increased sympathetic innervation or a reinforced α_2-adrenergic response of pancreatic islets or both accounts for the impaired secretory response to glucose observed in certain forms of Type II diabetes. The effect of sympathetic hyper-innervation has been studied in a transgenic mouse in which overexpression of nerve growth factor (NGF) was specifically targeted to beta cells [37]. Surprisingly, these mice have nor-

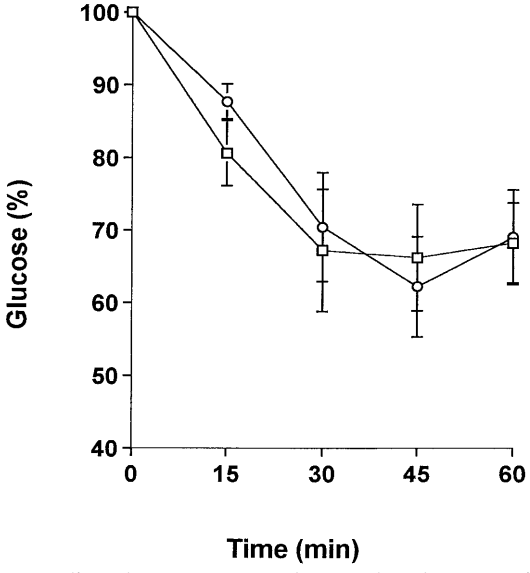

Fig. 6. Insulin tolerance test. Fed control and transgenic mice were injected intraperitoneally 0.75 IU of a soluble insulin (Humulin regular, Eli Lilly). Blood samples were taken from the tail vein of the same animals at the times indicated and glucose concentration measured as indicated in Methods. Results are means ± SEM of seven transgenic –○– and seven control (–□–) mice

mal regulation of glycaemia. Moreover, their islets showed enhanced insulin secretion in response to glucose and blunted response to the α2-antagonist, phentolamine, suggesting that the lack of incidence of hyper-innervation was primarily due to α_2-adrenoceptor desensitisation [38]. We reinvestigate the putative role of pancreatic α_2-adrenoceptors in the emergence of Type II diabetes by developing an animal model that overexpresses this receptor in beta cells.

The use of a chimeric gene containing the human insulin promoter fused to the α_2*C10AR* gene allowed us to obtain a transgenic mouse line in which α_2-adrenoceptor density in pancreatic islets was ninefold higher than in controls. Analysis of this model indicated that receptor overexpression did not cause any modification of insulinaemia or glycaemia. As already suggested [39], this lack of effect could be because the sympathetic system does not exert any tonic inhibition on insulin secretion under resting conditions. The possibility that compensatory mechanisms against the overexpression of the α_{2A}-adrenoceptor operate in these transgenic mice cannot, however, be ruled out. Injection of α_2-agonist UK14304 into control mice resulted in a 35% decrease in insulinaemia, whereas a 76% decrease was noted in transgenic mice. Thus, the extent of the insulin and glycaemic responses to UK14304 treatment was much higher in transgenic animals. The amplitude of the hyperglycaemic and hypoinsulinaemic response to UK14304 in control mice is consistent with that reported for this imidazoline compound [40]. Moreover, these effects are exclusively due to being occupied by agonists because of α_2-adrenoceptor they were efficiently blocked by non-imidazoline α_2-antagonists such as rauwolscine [40]. According to the experiments carried out on isolated islets, the reinforced action of the α_2-agonist in vivo in transgenic mice was probably the consequence of enhanced inhibition of insulin secretion. The molecular mechanisms by which α_2-adrenoceptors inhibit insulin release from beta cells are not understood. Several pertussis toxin-sensitive mechanisms, including inhibition of adenylyl cyclase, opening of ATP-sensitive K^+ channels and inhibition of L-type Ca^{++} channels contribute to the effect of α_2-agonists [41, 42]. Moreover, control of the late steps in the pathway of insulin vesicle exocytosis has also been shown [41, 42]. A recent study on insulinoma cells transfected with the cDNA encoding mouse α_{2A}-adrenergic receptor suggested that receptor overexpression reduces cellular insulin content and attenuates basal and stimulated insulin secretion [43]. In contrast, here we found that the glucose-stimulated insulin secretion was similar in islets isolated from control and transgenic mice. A stronger decrease in insulin release by transgenic islets than in control was, however, observed in the presence of the α_2-agonist. This indicates that receptor overexpression probably affects insulin secretion rather than insulin synthesis. Notably, intraperitoneal injection of glucose resulted in the emergence of glucose intolerance in transgenic mice. The difference between transgenic and control mice was not observed after injection of α_2-antagonist RX821002, suggesting it is primarily the consequence of enhanced α_2-adrenoceptor-mediated inhibition of insulin secretion after glucose injection. It is also of note that restoration of normoglycaemia was faster in RX821002-treated mice. The reason for this effect could be due to exclusive blockade of the α_2-adrenoceptors indicating that in spite of anesthesia the mice were undergoing stress because of repeated tail snipping. After intraperitoneal injection of saline solution control and transgenic mice both, however, showed similar blood glucose concentrations. Alternatively, this effect could be the consequence of the use of RX821002. At the concentration used in our study (10 mmol/kg), this imidazoline derivative is able to stimulate insulin secretion by a mechanism independent from interaction with α_2-adrenoceptors [44] and involving binding to imidazoline sites and blockade of ATP-sensitive K^+ channels [45].

The results obtained on this new model of transgenic mice show that a direct increased expression of α_2-adrenoceptors in beta cells can cause alterations in the regulation of insulin secretion, resulting in intolerance to glucose. This is consistent with previous studies reporting enhanced pancreatic α_2-adrenergic receptivity in rodent models of Type II diabetes and thus suggests that increased expression of inhibitory receptors could contribute to the aetiology of certain forms of this disease.

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