Content of Adenine Nucleotide Translocator mRNA in Insulin-Producing Cells of Different Functional States

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This study was undertaken to characterize the expression of the gene coding for the adenine nucleotide translocator (ANT) in the insulin-producing β-cell and to study any possible relationship between its expression and the functional state of the β-cell. Adult and fetal rat pancreatic islets were prepared and cultured under different conditions in vitro. The total RNA from these islets and from the insulin-producing RINm5F cells was isolated and analyzed by the Northern blot technique via a cDNA clone (pAAC-9) coding for the bovine ANT. We found that a 1600–base pair (bp) mRNA hybridizing to the pAAC-9 clone could be detected in RINm5F cells, and a 1450-bp mRNA was similarly observed in the islets. These sizes correspond well to previously reported forms of mRNA for the ANT observed in other tissues. When comparing the intensities of the pAAC-9 hybridizing bands of the different islet groups, it was observed that fetal islets contained less of this mRNA than adult islets. Furthermore, the content of the ANT mRNA in adult islets cultured at a high glucose concentration was increased compared with islets cultured at a low glucose concentration. Finally, streptozocin-treated islets, which display an impaired glucose-sensitive insulin release after 6 days in culture, also contained less of this mRNA than the control islets. We conclude that pancreatic islet cells express an mRNA that appears to be highly homologous to the bovine ANT and that the contents of this mRNA increases with the functional status of the β-cell. It is furthermore suggested that defects in the expression of this gene may be associated with impaired glucose sensitivity.
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The adenine nucleotide translocator (ANT) is an abundant, 30,000-M_r mitochondrial protein comprising ~6% of the total protein in this organelle (1–3). It forms a dimer that has four transmembrane regions, which creates a pore that allows transport of ADP and ATP across the inner membrane (4).

The cDNA sequences of the ANT have been completely or partially determined in human skeletal muscle (5), human fibroblasts (3), bovine heart (6,7) and *Neurospora crassa* (3). At the amino acid level, there is an 89% homology between the ANT of human skeletal muscle and human fibroblasts, showing that at least two different ANT genes exist in humans (5). That several ANT genes are expressed in mammals is further supported by the differences in electrophoretic mobility between bovine heart, kidney, and liver ANTs (8,9). Furthermore, the expression of the ANT gene has been shown to be regulated by external agents in human fibroblasts. More specifically, growth-promoting agents, serum, platelet-derived growth factor, and epidermal growth factor increased the ANT, i.e., mRNA content, whereas differentiating substances such as phorbol ester and retinoic acid decreased the contents (3).

Because the oxidative metabolism of the mitochondrion appears to play an essential role in insulin production in pancreatic β-cells (10) and because the ANT determines the rate of ADP-ATP flux between the mitochondria and the cytosol, we studied the expression of the ANT gene in insulin-producing cells and correlated this expression to the functional state of the β-cell, measured by insulin mRNA content, with particular attention to a model exhibiting impaired β-cell function.

RESEARCH DESIGN AND METHODS

Collagenase (EC 3.4.24.3) was obtained from Boehringer-Mannheim (Mannheim, FRG), and Hanks' balanced salt solution and calf serum were supplied by Statens Bakteriolo-

giska Laboratorium (Stockholm). Benzylpenicillin was from Astra (Södertälje, Sweden), and streptomycin was obtained from Glaxo (Greenford, UK). Culture medium RPMI-1640 and fetal bovine serum were obtained from Flow (Irvine, UK). Antibovine insulin serum was from Miles-Yeda (Rehovot, Israel). Crystalline mouse insulin and ^{125}I-labeled insulin were provided by Novo (Copenhagen). L-[4.5-^{3}H]leucine (sp act 130 Ci/mmol) and [α-^{32}P]dCTP (3000 Ci/mmol) were supplied by Amersham (Amersham, Buckinghamshire, UK). Econofluor was from New England Nuclear (Boston, MA), and cyanobromide-activated Sepharose 4B, Ficoll 400, and an oligonucleotide labeling kit were from Pharmacia (Uppsala, Sweden). Glyoxal, yeast tRNA, poly(A), providone, salmon sperm DNA, dithiothreitol, Dowex MR-3, and HEPES were from Sigma (St. Louis, MO). GeneScreen Hybridization Transfer Membrane was obtained from New England Nuclear. All other chemicals of analytical grade were from Merck (Darmstadt, FRG).

Preparation and culture of adult and fetal rat islets and RINm5F cells. Islets from adult rats were isolated on Ficoll gradients from collagenase-digested pancreases of male Sprague-Dawley rats weighing ~300 g (11,12). The islets were picked free from other pancreatic tissue by means of a braking pipette and were maintained free floating in tissue culture at 37°C in 95% air/5% CO_2. The culture medium was RPMI-1640 containing 10% calf serum, 100 U/ml benzylpenicillin, 0.1 mg/ml streptomycin, and 2 mM L-glutamine (13). The islets were cultured for 7 days, and the culture mediums were changed every 3rd day. For the experiments with different glucose concentrations, groups of islets were cultured in 3.3 or 28 mM glucose for 7 days.

For preparation of fetal rat islets, pregnant rats were killed by cervical dislocation on day 21 of gestation. Pancreases were excised from decapitated fetuses and minced for partial digestion with collagenase as described previously (14). The digests were seeded in attachment dishes and cultured for 3–7 days. After culture, the fetal islets were detached from the bottom of the dishes.

The insulin-secreting, continuously growing RINm5F cell line (15) was cultured in RPMI-1640 containing 10% fetal calf serum according to Swenne and Sjöholm (16). In preparation for experiments, the cells were scraped free from the dishes and washed in cold phosphate-buffered saline (PBS) (154 mM Na^+, 4 mM K^+, 140 mM Cl^-, and 10 mM HPO_4^{2-}, pH 7.4).

Treatment of islets with streptozocin (STZ). The islets were treated with STZ as described previously (17). Groups of 200 adult rat islets were preincubated in RPMI-1640 containing 5.6 mM glucose for 30 min at 37°C. STZ was dissolved in cold citrate buffer (10 mM, pH 4.5) within a minute before use, and this solution was added to the culture medium to obtain the required STZ concentration (0.55 mM). Islets were incubated with STZ for 30 min at 37°C in air and CO_2, and the incubation was terminated by the removal of the STZ-containing medium and the subsequent addition of culture medium. Control islets (treated with citrate buffer only) and STZ-treated islets were maintained in culture for an additional 6 days.

Isolation of total RNA and Northern blot analysis. Total RNA was prepared by the guanidinium method as described by Maniatis et al. (18). Groups of 1000–2000 adult or fetal rat islets or 2–4 × 10^6 RINm5F cells were briefly washed in cold PBS and subsequently transferred to 3 ml of 4 M guanidine isothiocyanate, 25 mM sodium citrate (pH 7), and 0.1 M β-mercaptoethanol. The guanidinium solution was placed on a 1.5-ml cushion of 5.7 M CsCl and was centrifuged at 40,000 rpm overnight in an SW-50 rotor. After removal of the supernatant, the RNA pellet was suspended in 1% sodium dodecyl sulfate, 10 mM Tris (pH 7.5), and 5 mM EDTA and extracted with phenol/chloroform/isoamyl alcohol (25:24:1, vol/vol/vol). After extraction, the RNA was precipitated overnight at −20°C in potassium acetate (0.07 M) and ethanol (70%, vol/vol). Some of the precipitated samples were subjected to oligo dT-cellulose chromatography for poly(A) selection, as described by Maniatis et al. (18). The remaining precipitates were washed with 70% ethanol and subsequently treated with 1 M glyoxal for 1 h at 50°C. Samples of 10–30 μg total RNA and 2–5 μg poly(A)-selected RNA were applied to 1% agarose gels and electrophoresed as described by Thomas (19). The RNA was transferred to GeneScreen membranes and attached by means of a 2-h incubation at 80°C. After prehybridization for 6 h at 42°C in 50% (vol/vol) deionized formamide, 5× sodium chloride–sodium citrate (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.5), 250 μg/ml sonicated salmon sperm DNA, 100 μg/ml poly(A), and 0.02% each bovine serum albumin, Ficoll, and povidone (19), the samples were hybridized at 42°C in the same solution with the pAAC-9 probe (6), which had been labeled with an oligonucleotide-labeling kit. After hybridization, the filters were washed and then exposed at −70°C to Hyperfilm-MP (Amersham) with an intensifying screen. The intensities of the bands thus obtained were determined by densitometry and expressed as optical density. The filters were rehybridized to insulin cDNA pRI-7 to assess the expression of insulin mRNA (20).

RESULTS

Autoradiographs of RNA blots showed that the labeled pAAC-9 probe hybridized to a 1600-bp RNA in RINm5F cells (data not shown). When comparisons were made with adult rat pancreatic islets, it was found that these cells expressed an mRNA of 1450 bp that hybridized to the pAAC-9 probe (Fig. 1*A*). The intensity of this band in islets obtained from rat fetuses (21 days gestation) and cultured for 3–7 days was found to be 67% lower than in control adult rat islets when a correction was made for differences in the amounts of total RNA applied (Fig. 1*A*, *lane 5*; Table 1). The content of this mRNA in adult rat islets treated with 0.55 mM STZ and then cultured for 6 days was also decreased compared with control islets (Fig. 1*A*, *lanes 1* and *2*; Table 1). In addition, islets treated with STZ exhibited decreased insulin release in response to glucose (data not shown). Finally, when the mRNA for ANT was measured in islets cultured in 28 mM glucose, it was found that these islets contained more of the 1450-bp mRNA than islets cultured in 3.3 mM glucose (Fig. 1*A*, *lanes 3* and *4*; Table 1). When the expression of insulin mRNA was determined in the same experiments, it showed the same relative changes as the ANT mRNA (Fig. 1*B*; Table 1), except that the fetal islets seemed to exhibit near-normal levels of insulin mRNA, although there was considerable variation between the different experiments.

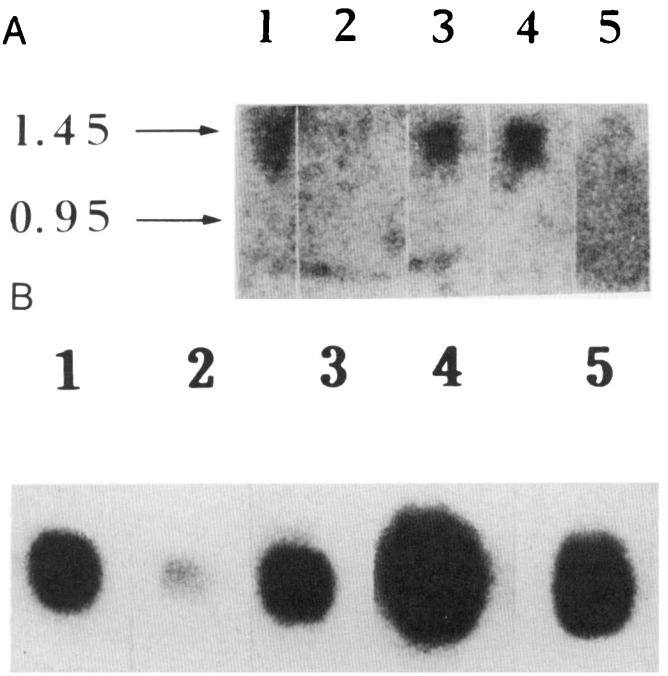

FIG. 1. *A*: RNA levels of adenine nucleotide translocator in islets cultured for 7 days in 11 mM glucose (*lanes 1, 2, and 5*), 3 mM glucose (*lane 3*), or 28 mM glucose (*lane 4*). Islets in *lane 2* had been treated with streptozocin on day 0. Islets in *lane 5* were isolated from fetal rats before culture, whereas all other groups were from adult rats. RNA was extracted, and hybridized radioactivity was determined autoradiographically. *B*: RNA levels of insulin in islets cultured for 7 days. Same blot as in *A* was rehybridized for insulin mRNA. *Lanes* are same as in *A*.

DISCUSSION

In this study, the expression of mRNA homologous to a cDNA coding for a fraction of the bovine heart ANT has been characterized in different insulin-producing β-cells. ATP production in pancreatic β-cells appears to be of fundamental importance in the regulation of insulin biosynthesis and release. Indirect evidence of a role of ATP in insulin mRNA transcription and insulin biosynthesis has been presented (21). Furthermore, it has been suggested that ATP participates in the coupling of metabolic and cationic events in the stimulus-secretion coupling (10). More specifically, the ATP content of the β-cell has been shown to increase in a sigmoidal fashion in response to glucose, and this increase was more pronounced in the cytosol than in the mitochondria (22). Increases in ATP content are thought to initiate insulin release by decreasing the activity of the ATP-dependent K^+ channel (23,24), an event that leads to depolarization and Ca^{2+} mobilization (25). The study by Yousufzai et al. (26) pointed to the existence of ANT in pancreatic islet cells and showed that inhibition of ANT leads to a decreased release of insulin. Furthermore, ANT has been implicated in regulation of Ca^{2+} mobilization and, therefore, also insulin release in that phosphoenolpyruvate generated in the glycolytic pathway could replace ADP in the translocase reaction and thereby decrease Ca^{2+} uptake in mitochondria (27). Our finding that insulin-producing cells express a 1450-bp mRNA homologous to the bovine ANT cDNA also points to a regulatory role of ANT in insulin production.

However, ANT mRNA is a messenger species of low abundance, and its hybridization to the bovine cDNA probe can be assumed to be far from optimal. For this reason, although as many as 2000 islets per lane were used, the signal/noise ratio of the Northern blots was low. This is further illustrated by comparing the autoradiographs (ANT mRNA) that were exposed to X-ray film for 8 days (Fig. 1*A*) with those (insulin mRNA) that were exposed to film for only 4 h (Fig. 1*B*). Despite the low signal/noise ratio of the ANT blots, it was feasible to detect major differences in the expression of the ANT gene. Thus, a possible functional role of the ANT mRNA was evaluated by examining pancreatic islets with different capacities for insulin synthesis and release. First, fetal rat islet, which has been shown to respond poorly to glucose with insulin release (14) despite the presence of a functional ATP-dependent K^+ channel (28), displays a sluggish and diminished increase in ATP in response to glucose (29). In our study, such islets contained less of the ANT mRNA than adult islets. Second, islets cultured for prolonged periods at a low glucose concentration displayed an attenuated insulin response compared with islets cultured at a high glucose level (30). Also, in this case, there was a positive correlation between the content of the ANT mRNA and the insulin-producing capacity. Finally, Eizirik et al. (31) reported that islets exposed to STZ and subsequently cultured for 6 days exhibit impaired glucose-sensitive insulin release. Using this model, we show that islets with long-lasting damage to the insulin-secretion apparatus contain less ANT mRNA. Two interpretations of these findings are possible. First, the mRNA hybridizing with the ANT cDNA may represent an ANT gene, which plays a primary role in the regulation of ATP production, Ca^{2+} mobilization, and ultimately, insulin production. Second, ANT mRNA is a nonspecific marker for mitochondrial activities, and changes in the contents of this mRNA are only secondary to other events.

In conclusion, we observed that insulin-producing cells express ANT mRNA that appears to be highly homologous

TABLE 1
Expression of adenine nucleotide translocator (ANT) mRNA and insulin mRNA in islet cells

mRNA	Control (11 mM glucose)	Streptozocin (11 mM glucose)	3.3 mM glucose	28 mM glucose	Fetal (11 mM glucose)
ANT	100	34 ± 10*	51 ± 6†	93 ± 25	33 ± 13*
Insulin	100	48 ± 2.4†	59 ± 10	125 ± 14	96 ± 68

Values are means ± SE of 3 experiments in percentages of control after normalization for the total amount of mRNA applied. Expression of ANT mRNA in islets was determined by densitometric scanning of Northern blots. The Northern blots used to determine ANT mRNA expression were rehybridized to insulin cDNA for determination of insulin gene expression.
*$P < .05$, †$P < .01$, vs. control by paired t test.

to bovine ANT. The expression of this mRNA seems to increase with the capacity of the cells to secrete insulin in response to glucose. These observations suggest a regulatory role of ANT in the pancreatic β-cell.

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