ACTA STEREOL 1994; 13/1: 3-8
PROC 6ECS PRAGUE, 1993
ORIGINAL SCIENTIFIC PAPER

MORPHOMETRIC EVALUATION OF THE PANCREAS OF GROWTH HORMONE-TRANSGENIC MICE

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ABSTRACT

The pancreas of MTbGH-transgenic mice exhibiting high levels of circulating growth hormone was analyzed by morphometry. Mice that did not inherit the foreign gene served as controls. Animals were examined at an average age of 11 weeks. The volume of the unfixed pancreas was measured by fluid displacement. Cavalieri's principle was applied to estimate the volume of the pancreas and the total volume of the pancreatic islets after paraffin embedding. The volume fraction of immunostained B-cells in the islets was determined following Delesse's principle. Total B-cell volume was obtained as the product of the fractional B-cell volume and the total volume of the endocrine pancreas. Values obtained for pancreas volume in paraffin-embedded tissue were 44% lower on the average than the values obtained for the unfixed organ. The volume of the pancreas, the total islet volume and the volume fraction of islets in the pancreas as well as the islet volume-to-body weight ratio were significantly increased in GH transgenic mice, whereas the pancreas-to-body weight ratio did not differ from control values. The volume fraction of B-cells in the pancreatic islets was similar between groups. The changes noted in the endocrine pancreas of GH transgenic mice encompass both an increase in total B-cell volume and a concomitant increase in the volume of other islet cells.

Key words: B-cell, Cavalieri's principle, growth hormone, pancreas, transgenic mouse.

INTRODUCTION

Various experimental strategies including hypophysectomy on the one hand, and the induction of increased serum growth hormone (GH) levels on the other hand, have been used to elucidate the functional relationship between GH and pancreatic islet cells in vivo. Hypersomatotropic rodent models have been established by exogenous administration of GH. Another model frequently used is the GH-prolactin-secreting tumor-bearing rat (for review cf. Davidson 1987, Press 1988). A novel approach to investigate hormonal effects in the context of the whole organism is provided by transgenic technology, which allows for the introduction of a known gene into the genome of an animal (Brem 1993). The transgene encoded heterologous protein is synthesized by the transgenic individual as an authentic self molecule. Transgenic mice expressing foreign GH genes have been established as valuable tools to study the consequences of long-term elevated circulating GH (Brem et al. 1989, Wanke et al. 1992).

The present study was carried out on transgenic mice expressing a bovine (b)GH encoding DNA sequence under the transcriptional control of the murine metallothionein 1 promoter (MT) in order to evaluate quantitatively the morphological changes of the murine pancreas under hypersomatotropic conditions.

ANIMALS, MATERIAL AND METHODS

MT-bGH transgenic mice (TM) were obtained as described in Wolf et al. (1993). Animals that did not inherit the foreign gene served as controls (CM). Six TM (4m, 2f) and eight CM (4m, 4f) were examined at a mean age of 77 ± 18 days (SD). Detection of the foreign fusion gene and quantitation of serum bGH levels have been carried out as described previously (Wanke et al. 1992). Animals were weighed to the nearest 0.1 g after a five hour fasting period and sacrificed after blood collection via orbital puncture under anesthesia by ether inhalation. The entire pancreas with attached spleen, stomach and intestine was excised, trimmed free of surrounding fat and peritoneal tissue, and weighed to the nearest 0.1 mg. Pancreas volume was measured with the submersion method of Scherle (1970) as described by Weibel (1979). The whole organ was then placed in a tissue capsule on a piece of foam-rubber sponge to avoid distortion during immersion fixation in 5% buffered formalin for 48 hours and embedded in Paraplast®. Cavalieri's principle was applied to estimate the volume of the embedded, i.e. shrunken pancreas as well as the volume of the pancreatic islets (Gundersen and Jensen 1987). First, the embedded organ was trimmed free of Paraplast® until it became clearly visible, and its length along the longitudinal axis was recorded. After positioning the first cut randomly within an interval of 1mm length at the splenic end of the pancreas, the organ was exhaustively sectioned perpendicular to its longitudinal axis into parallel slices of approximately 1mm thickness. Since the real distance between two cuts was likely to vary somewhat, the mean thickness of the slices ($\bar{t}$) was estimated by dividing the length of the organ by the number of sections, and $\bar{t}$ was used for volume estimation as described in Michel and Cruz-Orive (1988). These slices were placed with the right-hand cut-surface upwards in tissue capsules and reembedded in Paraplast®. From each slice, a series of histological sections for various staining procedures was cut at a nominal thickness of 3 μm. The first complete section from each block was stained with hematoxylin and eosin (H&E) and used for morphometric evaluation carried out on a Videoplan® image analysis system (Zeiss-Kontron, Germany) coupled to a microscope via a color video camera. The cross-sectional area of pancreas tissue was planimetrically determined on micrographs prepared from each histological section at an 11x final linear magnification. For calibration, an object micrometer (Zeiss, Germany) was photographed under the same conditions at the beginning of every set, and prints of all negatives in each series were made at a constant setting of the enlarger. Measurement of islet profiles was carried out on images displayed on a color monitor. In the H&E stained sections all unambiguously identifiable profiles were measured by tracing their contours with a cursor on the digitizing tablet of the image analysis system. For this purpose, a 25x objective was used providing a 850x final magnification. The coefficients of error (CE) for the Cavalieri estimates of pancreatic tissue and total islet volume (V_{islet}) were calculated according to Gundersen and Jensen (1987). A correction factor for shrinkage due to histological processing was calculated for each individual organ as the volume of the unfixed pancreas divided by the volume of the embedded pancreas. The volume fraction of islets $V_{V(islet/pancreas)}$ was calculated as the sum of cross-sectional areas of pancreatic islets divided by the sum of cross-sectional areas of the whole pancreas (Weibel 1979). Assuming the same extent of shrinkage for islets and the whole organ, the volume of pancreatic islets in the unfixed pancreas was calculated taking into account the individual

shrinkage. In four transgenic and four control mice, one section from each block was immunohistochemically stained for insulin using an indirect immunoperoxidase technique (Nakane and Pierce 1967) in order to estimate the volume fraction of B-cells ($V_{V(B\text{-}cell/islet)}$). Guinea pig-anti-porcine insulin and horseradish peroxidase conjugated rabbit-anti-guinea pig immunoglobulin antisera were purchased from DAKO, Denmark. Diaminobenzidine was used as chromogen. Specifity controls included the pre-absorption of the primary antiserum with an excess of porcine insulin (Sigma, Germany) and the omission of the primary antibody. The sections were counterstained with hematoxylin. For sampling of immunostained islet profiles, two parallel vertical lines on the monitor were employed. Starting from a random point which did not hit pancreas tissue, each section was examined as a series of parallel stripes at a 1400x final magnification using a 40x objective. Sampling of islet profiles was performed in every second stripe with the stripe width corresponding to 200 μm. The visual field was moved along the sampling lines, and islet profiles between the lines or touching the right hand line were sampled, while those touching the left hand line were excluded to give an unbiased sample (Gundersen 1977). The number of immunohistochemically stained islet profiles measured in each individual ranged from 41 to 60 in CM and 63 to 72 in TM. Both the profile area and the area of immunostained tissue were determined planimetrically and $V_{V(B\text{-}cell/islet)}$ was obtained by dividing the cumulative cross-sectional area of immunopositive tissue by the cumulative area of sampled islet profiles (Weibel 1979). Total B-cell volume ($V_{B\text{-}cell}$) was estimated as the product of $V_{V(B\text{-}cell/islet)}$ and V_{islet}. Means were compared by Wilcoxon's two-sided rank-sum test; $p<0.05$ was considered statistically significant.

RESULTS

Serum concentrations of bGH ranged from 193 to 717 μg/l in the TM. The mean body weight was significantly increased both in male and female TM, as compared to their non-transgenic counterparts. Pancreas weight as well as the volume of the organ before and after embedding were significantly increased in the TM, whereas the pancreas-to-body weight ratio remained almost unchanged (cf. Tbl. 1., Fig. 1.). Values obtained for the specific gravity of the pancreas averaged 1.08 mg/mm^3 (± 0.01 SD) and did not differ significantly between TM and CM. The volume of the embedded pancreas was estimated at 55 ± 4.6% (mean ± SD) of the volume of the unfixed pancreas in CM and at 58 ± 5.8% in TM - a difference, which was not statistically significant (cf. Fig. 1.).

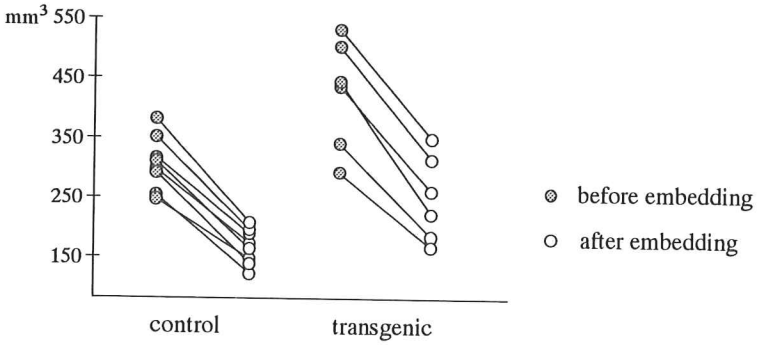

Fig. 1. Volume of the pancreas
Slope of lines connecting data points indicates individual shrinkage

Table 1: Parameters evaluated in control and transgenic mice
*: significant difference ns: no significant difference

			control		transgenic		
			mean	SD	mean	SD	
body weight	[g]		32.5	4.5	45.3	8.0	*
pancreas weight	[mg]		335	49.4	452	97.6	*
pancreas/body weight	[%]		1.04	0.16	1.00	0.11	ns
pancreas volume	[mm^3]	shrunken	169	29	249	72	*
		unfixed	308	45	424	92	*
V_{islet}	[mm^3]	shrunken	0.70	0.11	1.40	0.35	*
		corrected	1.28	0.17	2.38	0.42	*
$V_{V\ (islet/pancreas)}$	[%]		0.42	0.07	0.57	0.04	*
V_{islet} / body weight	[$x10^{-5}$]		3.96	0.53	5.29	0.37	*
$V_{B\text{-}cell}$	[mm^3]	shrunken	0.54	0.08	1.09	0.27	*
		corrected	0.98	0.16	1.88	0.34	*

The total islet volume, the volume fraction of pancreatic islets and the islet volume-to-body weight ratio were significantly increased in TM as compared to CM (cf. Tbl. 1. and Fig. 2.) The observed coefficient of variation (CV) of the total islet volume estimate was 16% between CM and 25% between TM when shrunken volumes were considered and corresponded to 13% among CM versus 18% among TM, when calculated with values corrected for shrinkage. The CE of the estimate of V_{islet} was 0.069 ± 0.012 (mean ± SD) in CM and 0.063 ± 0.019 in TM. The volume fraction of B-cells did not differ between TM and CM (cf. Fig.3.), while total B-cell volume was significantly increased in TM (cf. Tbl. 1.).

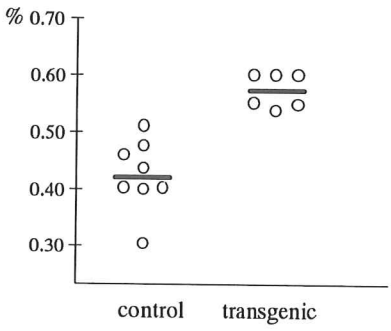

Fig. 2. Volume fraction (islet/pancreas)
individual value o
group mean —

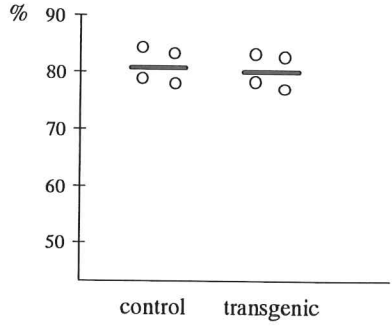

Fig. 3. Volume fraction (B-cells/islet)
individual value o
group mean —

DISCUSSION

In the present study, the volume of the pancreas and the pancreatic islets of CM and TM exhibiting high serum bGH levels was estimated. Only few studies exist where the total volumes of the exo- and endocrine mouse pancreas have been reported. Gepts et al. (1960) estimated the total volume of the endocrine pancreas in obese-hyperglycemic mice and non-obese littermates by measuring islet profiles in serial sections planimetrically and compared these data to those obtained by the method of Tejning (1947). Both methods provided similar results. The total islet volume in Bouin-fixed, paraffin-embedded tissue was estimated at 0.50 mm^3 in 4-month-old and at 0.67 mm^3 in 6-month-old non-obese animals. Hellman (1970) estimated the average total islet volume in paraffin-embedded pancreata from control mice at 0.79 mm^3 by counting large islet profiles in serial pancreatic sections taken at regular intervals. The method is based on the assumption that islets are spherical or ellipsoidal bodies. It was suggested as a rapid estimator of the total islet volume in humans and rodents, since measurements of large series of islet cross-sectional areas are avoided (Hellman 1959, Brolin and Hellman 1963). In the present study, an unbiased estimate of V_{islet} was obtained by evaluating only one histological section from each pancreatic slice produced by exhaustive systematic sectioning and following Cavalieri's principle. With sections taken at intervals of approximately one millimeter the CE of the estimate of total islet volume was sufficiently low. Values reported for $V_{V(B\text{-}cell/islet)}$ in various strains of mice range from 77% to 83% (Zeidler et al. 1989, Moreira et al. 1991). A comparison of the morphometric data obtained for the CM in this study with data from the literature requires consideration of methodological and biological variables. Despite these variables, the values reported for V_{islet} and $V_{V(B\text{-}cell/islet)}$ in the control mice of the studies mentioned above are in accordance with those for the CM in the present study. The mean specific gravity of the unfixed murine pancreas was found to be identical to the value for guinea pig pancreas as reported by Bolender (1974).

Values obtained for the volume of the embedded pancreas were 44% lower on the average than values obtained for the organ prior to fixation. However, the high amount of shrinkage with paraffin-embedding was not unexpected. An amount of 60% volume shrinkage due to fixation, paraffin-embedding and sectioning was reported for human lung (Weibel 1963). Mean linear shrinkage for rat kidney embedded in paraffin was estimated at 0.163 (Marcussen 1990). A volume reduction of approximately 41% may be calculated based on this value, which is comparable to the extent of shrinkage observed in this study. The estimate of total islet volume showed a smaller interindividual CV in CM and in TM when data corrected for shrinkage were used. Thus, neglecting the amount of individual shrinkage results in an overestimation of the CV among animals.

Mice expressing MTGH transgenes exhibit hyperinsulinemia (Wanke et al. 1992). The differences in $V_{V(islet/pancreas)}$ and the V_{islet}-to-body weight ratio between TM and CM indicate that the increase in V_{islet} in TM exceeds the increase in organ volume and overall body size. Since $V_{V(B\text{-}cell/islet)}$ in TM versus CM remained unchanged, it is concluded that the increase in V_{islet} is due to both an increase in $V_{B\text{-}cell}$ and a concomitant increase in other islet cells. Whether these changes are also reflected in an elevation of the serum levels of other islet hormones remains to be established.

ACKNOWLEDGEMENT

This work was supported by the Deutsche Forschungsgemeinschaft (He 1731/1-3, RW, WH) and the Studienstiftung des deutschen Volkes (EK).

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