Tissue Culture of Human Fetal Pancreas

Effects of Nicotinamide on Insulin Production and Formation of Isletlike Cell Clusters

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Human fetal pancreas (HFP) is a potential source of β-cells for transplantation to insulin-dependent diabetic patients. We have previously described a method for tissue culture of HFP that results in the in vitro development of isletlike cell clusters (ICCs) containing a minority of insulin-positive cells. Recently we found that nicotinamide, an inhibitor of poly(ADP-ribose) synthetase, induces an increased islet cell DNA replication both in vivo and in vitro. In this study, this culture technique was used to evaluate the effects of addition of 10 mM nicotinamide on HFP explants cultured in RPMI-1640 medium plus 10% human serum. ICCs developed in 11 of 19 consecutive cultures with nicotinamide increased the yield of ICCs by 40%. Also, the insulin content of ICCs increased ~50% with nicotinamide supplementation, although measurements of DNA indicated an unchanged number of cells in each ICC. Neither the rates of insulin release in response to 16.7 mM glucose plus 5 mM theophylline nor the (pro)insulin or total protein biosynthesis rates were affected by nicotinamide addition. The combined results of this study suggest that nicotinamide is useful for stimulating the formation of ICCs from HFP. *Diabetes* 38 (Suppl. 1): 168–71, 1989

Previous studies have shown that tissue culture of human fetal pancreas (HFP) results in the development of so-called isletlike cell clusters (ICCs) in vitro (1). The ICCs contained few insulin-positive β-cells and responded poorly to insulin secretagogues. However, after transplantation of ICCs to nude mice, an accumulation of endocrine cells within the grafts could be observed 8 wk later. In subsequent studies, we have found that supplementation of the culture medium with human serum (2) or human amniotic fluid (3) stimulated the formation of ICCs in vitro from HFP. When growth hormone was added to cultures supplemented with human serum, a higher yield of ICCs was achieved, and this was accompanied by an increased insulin content in the ICCs, suggesting that growth hormone may also stimulate the insulin production of explanted HFP cells (4).

A major obstacle for successful islet transplantation in insulin-dependent diabetes mellitus is to obtain a sufficient amount of islet tissue. However, if the growth of the tissue to be implanted could be markedly stimulated before implantation, the transplantation may have a better chance of success. One compound of great interest in this context is nicotinamide (NIC), because treatment of partially depancreatized rats with nicotinamide ameliorated their diabetes (5). Nicotinamide has also been found to ameliorate the spontaneous diabetes of nonobese diabetic mice (6) and to prevent the disease from recurring in islet-transplanted nonobese diabetic mice (7). In line with these findings, we have found that nicotinamide supplementation increased the DNA replication of cultured adult mouse pancreatic islets (8) and that treatment of mice increased the DNA replication in transplanted islets (9). In this study we examined the effect of nicotinamide on the formation and insulin biosynthesis and release of ICCs after explant culture of HFP. The contents of DNA and insulin of the explants were also evaluated.

MATERIALS AND METHODS

Culture of HFP. Over a period of 3.5 mo, 19 consecutively obtained HFP glands were explanted into tissue culture. The glands were collected at hospitals in Uppsala and Stockholm, Sweden, after legally approved abortions. All abortions were induced by prostaglandin administration and were usually completed within 24 h after induction. The use of human fetal tissue was in accordance with the principles expressed in the Declaration of Helsinki and was also approved by the local ethics committee. The crown-heel length of the fetuses ranged 13–26 cm (mean 17.0 cm), which corresponds to a gestational age of 12–24 wk.

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The dissection and collagenase digestion of the HFP were performed as described previously (1–4). After washing the digested HFP, the pelleted tissue was resuspended in 1 ml RPMI-1640 medium and subdivided between two culture dishes (Nunclon, 50 mm; Nunc, Roskilde, Denmark), each containing 4.0 ml RPMI-1640 and 0.5 ml heat-inactivated pooled human serum (HS) or RPMI-1640 + 10% HS + 10 mM nicotinamide (HS + NIC). The explanted tissue was incubated at 37°C in a gas phase of 95% humidified air/5% CO_2. The culture medium was initially changed after 48 h and subsequently changed every 24 h until day 7. Medium samples were collected for analysis of the insulin accumulation in the culture medium. On day 7, all ICCs present in the explant cultures were counted with the aid of a stereomicroscope.

(Pro)insulin biosynthesis, insulin release, and insulin and DNA contents of ICCs. On day 7, ICCs were easily harvested by gentle suction with a braking pipette. The methods for estimations of rates of (pro)insulin biosynthesis and total protein biosynthesis and of insulin release and insulin and DNA contents of the ICCs have been described in our preceding communications (1,3,4).

Statistical analyses. Results were calculated as means ± SE and compared via Student's *t* test for paired or unpaired samples. When a paired *t* test was performed, the values for the HS group were set to 100%, and the HS + NIC values were expressed as a percentage of the corresponding HS value. Statistical correlations were tested by linear regression via least-squares analysis.

RESULTS

Eleven of 19 explanted HFPs showed development of ICCs in culture. In 5 of the 8 experiments in which there was no formation of ICCs in vitro, the glands were dissected >1.5 h after the completion of the abortion. One additional gland was obtained from a fetus showing postmortem damage of the abdominal organs.

ICCs appeared in culture within 24 h after explantation, and most of them remained free-floating throughout the culture period. There was a gradually increasing tendency for the ICCs to attach to cells growing on the bottom of the culture dishes. The HS + NIC medium seemed especially to promote the growth of fibroblasts. Supplementation with 10 mM nicotinamide to the culture medium increased the yield of ICCs by ~40% (Table 1). There was a linear correlation between the number of ICCs formed and the crown-heel length of the fetuses for the explants cultured with HS + NIC ($r = .73$, $P < .05$) but not for the explants cultured with HS alone ($r = .57$, $P > .05$). The size of the ICCs formed in the two media did not differ, as evidenced by their similar DNA contents (Table 1). However, HS + NIC increased the insulin content of the ICCs by >50%. When the total DNA content of the ICCs per explant was calculated, we found that HS + NIC increased the total DNA content by ~40%, and the corresponding value for the total insulin content more than doubled.

The insulin accumulation in the culture medium varied markedly among the individual explanted HFPs. The insulin accumulation per explanted half pancreas per 24 h during the first 2 days of culture was 1210 ± 412 ng ($n = 10$) from explants cultured with HS and 1510 ± 507 ng ($n = 11$) from the explants cultured with HS + NIC. For both types of cultures, there was a strong linear correlation ($P < .001$) between the insulin accumulation during the first 2 days of culture and the crown-heel lengths of the fetus (Fig. 1). Furthermore, the slope of the regression line was significantly steeper ($P < .01$) for the explants cultured in the presence of HS + NIC. The intercept with the abscissa was similar for the two slopes, i.e., at a crown-heel length of ~14.3 cm. However, during the subsequent 7-day culture, there was a progressive decline in insulin accumulation in medium in both groups (Fig. 2). After ~5 days, the insulin in medium had reached a plateau level of 25% of the initial insulin accumulation.

TABLE 1
Characteristics of isletlike cell clusters formed in vitro during culture of human fetal pancreas explants

	RPMI-1640 supplement			
	HS	*n*	HS + NIC	*n*
Number of ICCs formed*	357 ± 109	11	502 ± 113†	11
DNA content (μg DNA/10 ICCs)	0.39 ± 0.05	11	0.34 ± 0.03	11
Insulin content (ng insulin/10 ICCs)	3.5 ± 0.5	11	5.4 ± 1.2†	11
Total ICC DNA content* (μg DNA)	12.6 ± 4.2	11	17.5 ± 4.0†	11
Total ICC insulin content* (ng insulin)	142 ± 58.6	11	315 ± 126‡	11
(Pro)insulin biosynthesis (10^3 dpm · 10 $ICCs^{-1}$ · 2 h^{-1})				
1.67 mM glucose	1.3 ± 0.56	9	1.5 ± 0.5	11
16.7 mM glucose	1.3 ± 0.59	10	1.2 ± 0.5	11
Total protein biosynthesis (10^3 dpm · 10 $ICCs^{-1}$ · 2 h^{-1})				
1.67 mM glucose	25 ± 7.3	9	23 ± 5.4	11
16.7 mM glucose	27 ± 7.7	10	20 ± 4.6	11
Percentage (pro)insulin of total protein biosynthesis (%)				
1.67 mM glucose	4.8 ± 0.8	9	5.9 ± 1.2	11
16.7 mM glucose	4.0 ± 0.8	10	5.2 ± 1.0	11

Values are means ± SE; *n* is number of observations. HS, 10% human serum; HS + NIC, 10% human serum + 10 mM nicotinamide; ICC, isletlike cell cluster.
*Values are from data per half pancreas, because the individual explanted glands were equally divided between culture dishes supplemented with either HS or HS + NIC.
†$P < .05$ and ‡$P < .01$, for a chance difference vs. HS with Student's paired *t* test.

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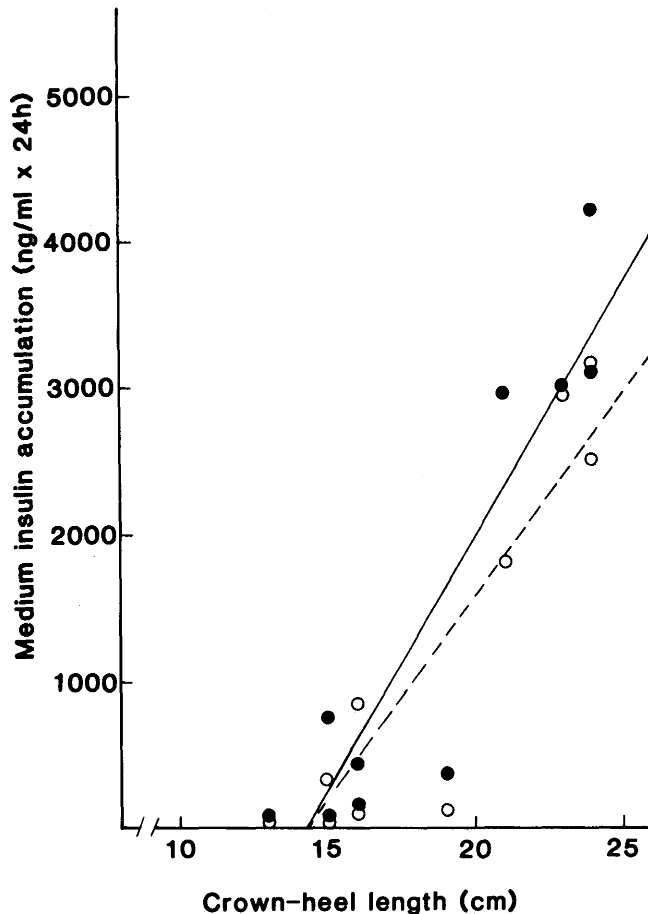

FIG. 1. Relationship between fetal crown-heel length and medium insulin accumulation during first 2 days of culture, for 10 human fetal pancreas explants maintained in medium supplemented with human serum (HS; ○) or with human serum plus nicotinamide (HS + NIC; ●). Regression lines for explants: *broken line*, cultured with HS, $y = 288x - 4145$ ($r = .91$); *solid line*, cultured with HS + NIC, $y = 357x - 5125$ ($r = .92$).

The rates of (pro)insulin biosynthesis were equal in the ICCs obtained after culture with HS or HS + NIC (Table 1), as were total protein biosynthetic rates. Glucose failed to stimulate either (pro)insulin or total protein biosynthesis in any of the groups of ICCs. The contribution of (pro)insulin to the total protein biosynthesis was 4–6% at either 1.67 or 16.7 mM glucose in both groups of ICCs. The insulin secretion in batch-type incubations was low even after stimulation with 16.7 mM glucose + 5 mM theophylline, and NIC did not induce increased insulin release compared with ICCs developed with the HS supplement only (data not shown).

DISCUSSION

This study shows that culture of HFP explants in the presence of NIC increases the yield of ICCs, as well as their total DNA and insulin content. The observation that the cellular number per ICC, as evidenced by DNA content, was not increased after culture in the medium supplemented with HS + NIC indicates that the β-cells maintained in this medium contained more insulin per β-cell than those in the HS medium. Because the rates of (pro)insulin biosynthesis of the ICCs cultured with HS or HS + NIC were equal, the higher insulin content in the latter group probably reflects an increased capacity of the β-cells cultured in the presence of NIC to store their synthesized insulin. A similar observation was recently made for HFP explants supplemented with growth hormone during culture (4). On the other hand, NIC failed to stimulate the capacity of the ICCs to respond to a secretory stimulus with increased insulin secretion, as was the case after growth hormone supplementation.

The mechanism by which NIC stimulated the formation of ICCs is unknown. NIC is a known inhibitor of poly(ADP-ribose) synthetase activity in several cells, and it has been shown that ADP-ribosylation reactions affect several cellular functions, including cell replication (10). In general, an increased poly(ADP-ribose) synthetase activity would lead to a restricted DNA replication. In this context, Okamoto (11) has proposed that poly(ADP-ribose) synthetase inhibitors could counteract a suppression of β-cell DNA synthesis and thus stimulate a β-cell replication. However, researchers have also suggested that poly(ADP-ribose) synthetase inhibitors may exert many different effects, some of which can be attributed to reactions other than reductions in cellular poly(ADP-ribose) concentrations alone (12).

The calculated regression lines between the initial medium insulin accumulation and the length of the fetuses give further information on the importance of the gestational age for the production of ICCs in culture (Fig. 1). Thus, with our culture

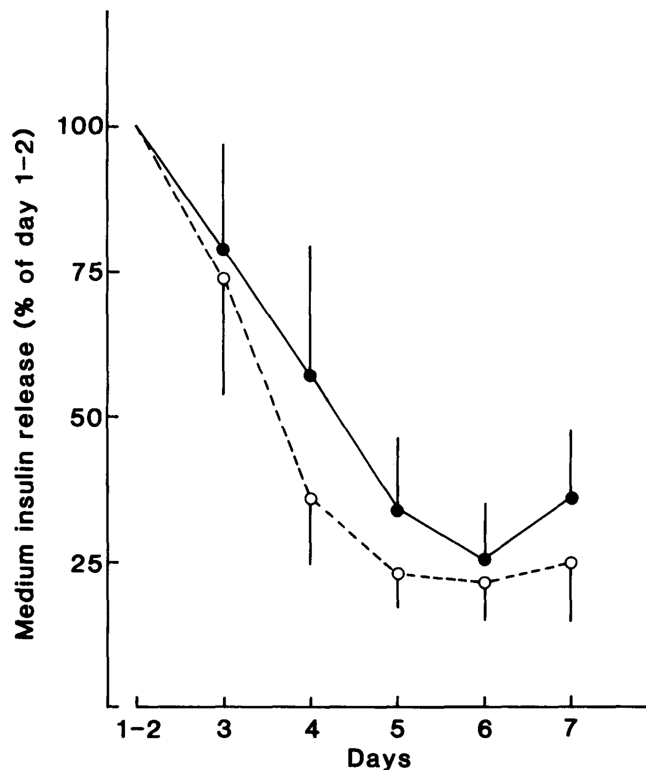

FIG. 2. Insulin release into medium during culture period. Insulin accumulation during first 2 days was 1210 ± 412 ng/24 h when medium was supplemented with human serum (●) and 1510 ± 507 ng/24 h when it was supplemented with human serum plus nicotinamide (○). Values are means ± SE for 10 human fetal pancreas explants.

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technique, ICCs formed from fetuses with crown-heel lengths <14 cm, corresponding to a gestational time of 14–15 wk, do not produce and release insulin. Previous studies have shown that the youngest fetuses in which scattered endocrine pancreatic cells can be identified were aged 8–9 wk (13,14). About 4 wk later, clusters of β-cells organized into primitive islets were observed, and at a gestational age of 20 wk, most endocrine cells were located in a more mature form of islets. However, the technique we used for production of ICCs is different from standard methods for islet isolation. The crucial step of our technique seems to be the reaggregation of dispersed fetal pancreatic cells, which leads to the formation of ICCs. Only a few of these cells stain specifically for insulin after several days in culture, and our study is an attempt to define procedures for stimulating both the differentation and replication of β-cells. If, however, fetuses of more advanced gestational ages were used, the maturation of the endocrine pancreatic part might have proceeded to a stage where this culture method is less feasible.

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