ecdysteroids on maturation of female beetles could recently be ruled out[2].

In *P. nigrita,* photoperiods are only perceived by means of the compound eyes[7]. In an unknown way the day-length stimulates the neurosecretory cells (NSC) of the pars intercerebralis to produce and release paraldehydfuchsin (PAF) stainable material. This neurosecretion is transported along the nervi corporis cardiaci I (NCC) to the corpora cardiaca and may regulate the CA activity. In SD beetles this PAF positive material is present in large amounts in the brain; however, there is little transport along the NCC I. In females maturing after transfer from SD into LD, transport of neurosecretion could be demonstrated.

It can be concluded that SD photoperiods mainly stimulate activity of the NSC in the pars intercerebralis while LD allows transport of this accumulated material. Moderate LD (e.g. LD 16/8) stimulate both production and release of neurosecretion.

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Hormone content of mouse pancreatic islets subjected to different in vivo and in vitro functional demands[1]

S. Pääbo, G. Lundqvist, B. Petersson and A. Andersson[2]

Department of Medical Cell Biology, University of Uppsala, Box 571, S-751 23 Uppsala (Sweden), and Department of Clinical Chemistry, University Hospital, Uppsala (Sweden), 2 March 1981

Summary. Mice treated for 4 days with tolbutamide displayed decreased serum glucose values with a concomitant decrease of their islet insulin content. Mouse islets cultured for 1 week at a low (3 mM) or a high (28 mM) glucose concentration contained less insulin than non-cultured islets and islets cultured at a medium (11 mM) glucose concentration. All groups of cultured islets contained more glucagon than non-cultured islets. The somatostatin content of high- and medium-glucose cultured islets was higher than that of freshly isolated islets.

During recent years much attention has been paid to pancreatic B-cells subjected to widely varying functional demands. Such experiments have been performed in vivo by starving experimental animals[3] or by treating them with, for example, sulfonylureas for a prolonged time period[4]. Alternatively, islet culture techniques have been used for assessing the in vitro effects of different supplementations of the culture media, i.e. glucose or amino acids[5,6]. The present study was undertaken to determine the hormone (insulin, glucagon and somatostatin) content of islets isolated from starved mice or mice treated for several days with tolbutamide. Furthermore, we determined the hormone content of islets maintained in culture for 1 week at different glucose concentrations.

Materials and methods. Collagenase (type CLS, 150–200 U/mg) was obtained from Worthington Biochem. Corp., Freehold, New Jersey, USA. Tissue culture medium (RPMI 1640 either glucose-free or containing 11 mmoles/l glucose) was supplied by Flow Laboratories Ltd, Irvine, Ayrshire, Scotland. Calf serum and Hanks' solution were obtained from Statens Bakteriologiska Laboratorium, Stockholm, Sweden. ^{125}I-insulin and ^{125}I-Tyr^{1}-somatostatin were from New England Nuclear, Dreieich, West Germany. Anti-insulin serum (code No. 65-101; lot No. GP20) was from Miles Laboratories, Inc., Elkhart, Ind., USA. ^{125}I-porcine glucagon, anti-porcine glucagon rabbit serum (K4023), crystalline mouse insulin and crystalline porcine glucagon were all obtained from Novo A/S, Bagsvaerd, Denmark. The anti-somatostatin serum used (R 141) was a gift from Dr R.P. Elde, University of Minnesota, Minneapolis, USA, and has been characterized elsewhere[7,8]. Crystalline ovine somatostatin was from Beckman, Geneva, Switzerland. Tolbutamide (Rastinon®) was purchased from Hoechst, Frankfurt, FRG. Other chemicals used were all of analytical grade.

Adult, male NMRI-mice (Anticimex, Sollentuna, Sweden) were used throughout the study. The animals were killed at about 08.00 h by decapitation and pancreatic islets isolated by collagenase digestion as described previously[9]. The islets were either transferred to culture dishes (see below) or directly homogenized by sonication for 30 sec in 500 µl acid ethanol (15 ml 12 moles/l HCl in 70% ethanol) and extracted over-night at +4 °C. Extracts were stored at −20 °C before the hormone assays.

For the in vivo experiments tolbutamide was injected i.p. as a 5% (w/v) solution (Rastinon®) to give a dose of either 125 or 300 mg/kg b.wt twice a day for 4 days. Control mice were injected with a 0.9% (w/v) sodium chloride solution. Another group of mice was starved with free access to drinking water for 60 h. Immediately before decapitation the orbital vein plexus was punctured and blood drawn for determination of the glucose and insulin concentrations.

For the in vitro experiments pancreatic islets were isolated from mice, which had been starved over-night prior to experimentation. The islets were transferred to Petri dishes for free-floating culture[3] at a glucose concentration of 3, 11 and 28 mmoles/l, respectively. The culture medium consisted of RPMI 1640 supplemented with 10% (v/v) calf serum and antibiotics (100 U/ml penicillin and 0.1 mg/ml streptomycin). The dishes were incubated at +37 °C in a gas phase consisting of 5% CO_2 in humidified air and the medium was changed on the 3rd and 5th days of culture. After 7 days the islets were harvested, homogenized and extracted as described above.

Insulin, glucagon and somatostatin contents of the islet extracts as well as the serum insulin concentrations were all determined by means of radioimmunoassay procedures[7,8,10,11], separating free and antibody-bound hormone by ethanol precipitation. Serum glucose was determined using a Beckman Glucose Analyzer 2 (Beckman Instruments, Inc., Fullerton, Calif., USA).

Results are given as means ± SEM with the number of experiments given in parentheses. For statistical analyses Student's t-test was used.

Table 1. Serum glucose and insulin concentrations and islet hormone contents of starved or tolbutamide-injected mice

Treatment before islet isolation	S-glucose (mmole/l)	S-insulin (ng/ml)	Islet hormone content (ng/25 islets) Insulin	Glucagon	Somatostatin
Controls	9.3±0.3 (12)	3.0±0.6 (12)	1206±76 (18)	40±3 (18)	22±1 (18)
Starvation for 60 h	3.2±0.4*** (12)	0.7±0.4** (12)	1090±77 (24)	34±2 (24)	22±1 (24)
Tolbutamide i.p. 125 mg/kg b.wt for 4 days	6.6±0.4*** (12)	2.7±0.3 (11)	992±57* (17)	36±4 (17)	21±1 (17)
Tolbutamide i.p. 300 mg/kg b.wt for 4 days	6.8±0.6** (12)	2.6±0.6 (12)	932±46** (18)	35±4 (18)	20±2 (18)

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ denote statistical significances of the differences between experimental groups and controls.

Table 2. Hormone contents of non-cultured or cultured islets

Islet specimen	n	Islet hormone content (ng/25 islets) Insulin	Glucagon	Somatostatin
Freshly isolated (starved over-night)	8	810±116	33±4	14±2
Culture at 3 mmoles/l glucose	8	131±28***	82±9***	20±4
Culture at 11 mmoles/l glucose	8	854±129	88±10***	37±3***
Culture at 28 mmoles/l glucose	8	508±80*	90±12***	30±4***

* $p < 0.05$ and *** $p < 0.001$ denote statistical significances of the differences between cultured and freshly isolated islets.

Results and discussion. In the in vivo experiments starvation induced a pronounced decrease of both the serum glucose and insulin concentrations (table 1). Yet, there was no effect on the hormone content of the isolated islets. It thus seems as though mouse islets are more resistant than rat islets to the effects of fasting on their content of insulin and somatostatin[12]. The fact that the insulin content remained unchanged is, however, not surprising, since the biosynthetic rate is only marginally impaired during fasting[13] and the rate of insulin release markedly decreased. Therefore, the occurrence of a decreased insulin content of the islets of the tolbutamide-treated mice seems logical, considering their normal serum insulin concentrations and the impaired rate of islet insulin biosynthesis[4]. It is noteworthy that the serum glucose concentrations of the tolbutamide-treated mice were decreased, despite the normal insulin concentration, which suggests the involvement of extrapancreatic factors in the long-term effects of sulfonylureas.

The present in vitro experiments clearly show that marked differences of the islet hormone content can be induced by exposing the cultured islets to different glucose concentrations (table 2). Thus, the insulin content of islets cultured at a low glucose concentration (3 mmoles/l) was extensively reduced, concomitantly with decreased rates of insulin secretion and biosynthesis[14]. Moreover, culture in 28 mmoles/l glucose produced a decrease of the islet insulin content, which, however, was less extensive than that we observed earlier for islets cultured in medium TCM 199[9]. This finding and the unchanged insulin content of islets cultured in the standard RPMI 1640 medium (11 mmoles/l glucose) could, at least partly, be due to the low calcium concentration of this particular medium.

All groups of cultured islets contained more glucagon than the freshly isolated ones. We recently showed that the glucagon content of islets cultured in RPMI 1640 was higher than that of islets cultured in TCM 199 or other media[15]. This probably explains why, in this study, culture increased the glucagon content of the islets, whereas culture in TCM 199 was found to result in a slight decrease of the glucagon content[16]. Common to both studies was, however, the lack of effect of the glucose concentration of the culture medium on the glucagon content. This finding gives further support to the view that mouse A_2-cells are relatively refractory to changes of the extracellular glucose concentration.

The somatostatin content of the islets cultured at the 2 higher glucose concentrations was higher than that of the freshly isolated ones. This was, however, not the case for the low-glucose cultured islets. It is thus tempting to speculate on a regulative role of glucose on the islet somatostatin content. In support of this notion, islets of streptozotocin-treated rats, with hypoinsulinemia and hyperglycemia, have a high content of somatostatin[17]. This also implies that there is no simple correlation between the extracellular insulin concentration and the somatostatin content of the islets.

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2 To whom all correspondance should be addressed.

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