Effects of pregnancy on the glucose-induced insulin release from cultivated pancreatic islets of the rat[1]

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Summary. 7-day-cultured islets from pregnant Wistar rats released at 5.6 mM glucose significantly more insulin than islets from nonpregnant rats, whereas in vivo this heigthened glucose sensitivity is lost 48 h post partum.

It was reported that insulin secretion is increased in pregnancy[2-4]. During pregnancy, the islets are more glucose-sensitive regarding insulin release and proinsulin/insulin biosynthesis[5-7]. However, all investigations with isolated islets were performed immediately after islet preparation and the experimental results may reflect a changed metabolic pattern, which was induced in vivo and which persisted in vitro several h after the isolation of the pancreatic islets.

It was therefore of special interest to investigate such metabolically adapted islets for a long time in vitro to prove whether the increased secretory response to glucose is maintained or is lost under culture conditions.

For this purpose, islets from 10 nonpregnant, 10 rats 48 h after parturition and from 15 pregnant (days 19–20) Wistar rats of comparable age (3–4 months) were used. After decapitation of fed animals, heparinized blood was collected and plasma glucose using Beckman glucose analyzer and plasma immunoreactive insulin were determined[8]. The islets were aseptically isolated by a collagenase (Worthington Biochemical Corp., USA, lot No. CLS 44 EO50) method as described in detail[9,10].

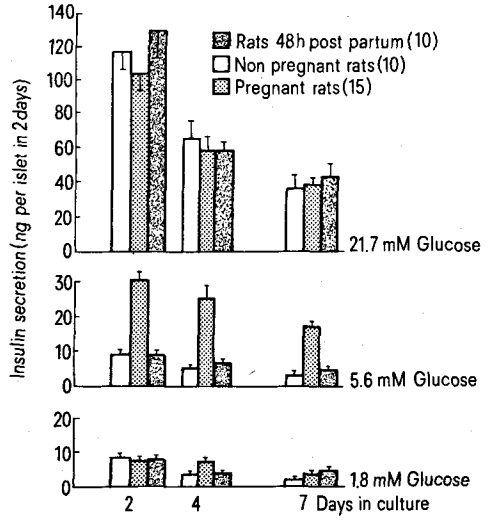
The isolated islets were collected in culture medium (TCM 199, Difco, USA, containing 5.6 mM glucose after mixture with 5% fetal calf serum and 5% heat-inactivated calf serum). Groups of 10 islets were cultivated in 2 ml of the same culture medium containing either 1.8, 5.6 or 21.7 mM glucose in glass petri dishes at 37 °C in a humidified atmosphere (95% air, 5% CO_2). Insulin in islets and their culture medium was assayed radioimmunologically[11].

The characteristics of animals and their islet insulin content is given in table 1. The plasma glucose is lowered in pregnant compared to nonpregnant rats and their insulin content of plasma as well as in islets is enhanced in accordance with Green and Taylor[6] and Saudek et al.[4]. 48 h post partum all data are comparable with those of nonpregnant rats (table 1). Furthermore pregnancy exerts an effect on the islet secretory process itself, the glucose threshold concentration is lowered, that means that the sensitivity of the B-cell to glucose is enhanced[6]. Our results in the figure show that the secretory response of islets from nonpregnant and pregnant rats to substimulatory (1.8 mM) and to high (21.7 mM) glucose concentration is comparable, but in the physiological range (5.6 mM) islets from pregnant rats released significantly ($p<0.01$) more insulin than the controls. Generally glucose concentrations up to 5.6 mM evoked an unstimulated insulin secretion from islets of nonpregnant rats, in short-term incubations[6,12] as well as under culture conditions (figure)[13]. The stimulatory effect of 5.6 mM glucose compared to 1.8 mM glucose in pregnant rat islets is maintained for 7 days (figure), even if the insulin content is diminished

Table 1. Characteristics of Wistar rats and their islets

Wistar rats	Plasma glucose (mg/100 ml)	Plasma insulin (ng/ml)	Islet insulin content (ng per islet)
Nonpregnant	136 ± 2.3	3.4 ± 0.4	71.5 ± 5.5
19–20 days pregnant	106 ± 5.2[a]	4.1 ± 0.4[b]	146.0 ± 12.6[a]
48 h post partum	127 ± 5.3[a]	2.8 ± 0.3	71.8 ± 5.8

Mean values ± SEM of 10 nonpregnant rats, 15 pregnant rats and 10 rats 48 h post partum.
[a]$p < 0.01$, [b]$p < 0.05$; effect of pregnancy.



Insulin secretion (ng per islet in 2 days) depending on the glucose concentration and on the age of culture.

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Table 2. Insulin content of Wistar rat islets (ng per islet) after 7 days of cultivation

Glucose during cultivation (mM)	Insulin content (ng per islet) 19–20 days pregnant rats	48 h post partum
1.8	14.5 ± 1.2	14.2 ± 2.5
5.6	26.9 ± 2.3	22.4 ± 2.3
21.7	43.4 ± 3.3	41.4 ± 2.8

Mean values ± SEM of 15 pregnant rats and 10 rats 48 h post partum.

as in islets from rats 48 h post partum (table 2). In vivo secretory pattern of islets from pregnant rats is normalized 48 h post partum (figure), but in vitro we observed a long-term effect, although the islets are removed from their physiological milieu and the islets cannot turn back as they do in vivo quickly.

The secretory response of islets from pregnant rats to 5.6 mM glucose is comparable with that of sand rat islets, which are also characterized by a lowered threshold concentration for glucose-induced insulin release found in freshly isolated as well as cultured islets, although the insulin content and release of sand rats are quickly exhausted by a glucose challenge in vitro[14].

But the ability of 5.6 mM glucose to stimulate the insulin release is maintained in islets of both species evoked by a short state (pregnancy) or a probably permanent state (species specificity) in sand rats.

We postulate that in vitro the insulin release during the long-term glucose challenge at high glucose concentration also depends on the capacity of the insulin net production (which can explain the continuous drop in content and secretion at 21.7 mM) but the recognition of the stimulus is adjusted in vivo and persists in vitro. The biochemical background of the hyperactivity of islets of pregnant rats is uncertain at present. It could be in connection with an enhancement of adenylate cyclase as well as of cellular cAMP content[15], or an alteration of a hypothetical receptor for glucose[16].

Further investigations are in progress to characterize islets adapted to different metabolic stages in vivo with regard to their behaviour under defined culture conditions in vitro.

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PRO EXPERIMENTIS

A low cost device for increased analytical capacity in gas chromatography[1]

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Summary. A simple device for automatic shut-down of a gas chromatograph is described. The device increases the analytical capacity by one sample a day, which is of interest when the retention times are of the order of several hours.

In gas chromatographic analysis, one often encounters the problem of long retention times. This will seriously limit the number of analyses that can be performed during normal working hours. One way to overcome this problem is to use a gas chromatograph equipped with an automated injector and a sample magazine large enough to contain samples for a whole night's continuous run. The prerequisite is, however, that all samples can be analyzed with the same instrument settings (temperature, amplification, etc.). If, however, the different samples require different settings, it will not be possible to use an automated gas chromatograph, unless it is controlled by a program unit with the capacity to store the different parameter values for each sample.

A time-saving compromise between manual and automated control would be a device that shuts down the instrument(s) after the last analysis of the day, without any technician having to be present. We have constructed a simple device with this function. It consists of a spring-type timer (Elektriska Instrument AB, Stockholm, type KS-50R), which will switch on or off the current fed to it at a given, preset time (0–6 h). In our case it has been wired into the circuits of the gas chromatogram recorder control so as to switch off the chart drive motor and the servo system of the recorder pen, leaving the amplifier live in the 'stand-by' state. The timer switch can be bypassed by means of the 'Hold-Timer' switch shown in the circuit diagram. With this switch in the 'Hold' position, the recording will go on until switched off manually. Just before leaving for the day, the technician sets the instrument controls properly and injects the sample. With the timer switch in the 'Timer' position, the required time for the analysis is preset and the gas chromatograph left on its own. After the required time, the chart drive and the pen servo system are switched off and the recording terminated.

With the simple, low-cost device described, it is possible to perform one analysis more a day without having to let the chart paper run all night or, even more expensive, having a technician present to switch off the recorder at the end of the run. The basic device described can easily be modified for use with any other apparatus, e.g. incubation baths, ovens, etc., by the use of adequate relay circuits controlled by the timer.

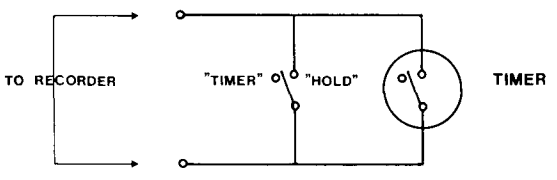

Diagram of the timer circuit described in the text.

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