Insulin secretion and (pro)insulin biosynthesis of cultured mice islets after glibenclamide loading in vitro[1]

Brigitte Ziegler, Barbara Wilke, M. Ziegler, S. Schmidt and H. Zühlke

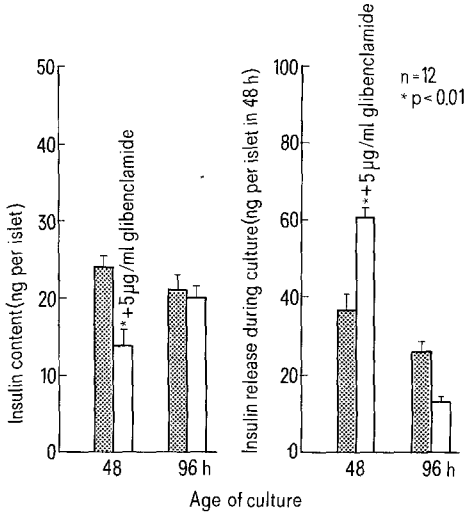
Central Institute for Diabetes "Gerhardt Katsch", DDR-2201 Karlsburg (German Democratic Republic), 18 June 1979

Summary. Isolated islets from C57Bl/6J mice exposed to 10 mmoles/l glucose supplemented with 5 µg/ml glibenclamide for 48 h released significantly less insulin in the subsequent short-time incubation than *untreated* controls (without glibenclamide), whereas insulin biosynthesis was not suppressed by glibenclamide treatment.

Sulfonylureas, which are stimulators of insulin release, restore blood glucose to normal values in some diabetics not requiring insulin. Chronic treatment of healthy animals, however, provoked a suppression of insulin release following a glucose loading in vivo and in vitro[2–6]. This persistent effect on insulin release was not found after exposure of isolated rat islets to glibenclamide for 2 h, whereas the insulin biosynthesis was influenced[7]. Since the treatment of animals requires days or weeks and the incubation in buffer for 2 h is probably a too short period for inducing this persistent effect, we have performed the following experiments with isolated islets in tissue culture to elucidate whether 2 days in vitro are sufficient to produce this persistent effect of glibenclamide as described after the treatment of animals. Furthermore, the question arises as to whether cultivation in the presence of glibenclamide also influences insulin biosynthesis in the following short-term incubation.

Material and methods. Collagenase-isolated islets of standard pellet chow fed C57Bl/6J mice weighing 25–30 g were used for the experiments. All steps were performed aseptically. In brief, 2 mice pancreata were digested by shaking with 5 ml collagenase solution, (containing 10 mg collagenase, 200 U/mg CLS IV 46K280P [Worthington, Biochem. Corp. Freehold, New Jersey, USA] and 10 mg bovine serum albumin [Behring Werke, Marburg, FRG] in 5 ml Hank's salt solution) for 8 min at 37 °C, washed with Hank's salt solution and isolated in TCM 199 containing 5 mmoles/l glucose, 5% fetal calf serum and 5% calf serum as previously described in detail[8]. Groups of 15 islets were cultivated in glass petri dishes containing 2 ml TCM 199 including serum (see above) and 10 mmoles/l glucose only (control) or, additionally, 5 µg/ml glibenclamide (test). These islets were placed in a humidified atmosphere (air with the addition of 3% CO_2) for 48 h at 37 °C. One petri dish of each group (control or glibenclamide treated) was taken for short term incubation: "labelling period" (2 h) at 37 °C, the remainder were cultivated in 10 mmoles/l glucose for a subsequent period of 48 h without glibenclamide. Washing of islets was performed between cultivation and short-term incubation with Krebs-Ringer bicarbonate buffer or TCM 199 as used for the subsequent culture period (10 mmoles/l glucose). The labelling period was carried out with 10 or 20 mmoles/l glucose, 20 µCi of ^{3}H-leucine (sp. act.: 56 Ci/mmole) from Amersham with groups of 5 islets each in 200 µl Krebs-Ringer bicarbonate buffer containing 1 mg/ml bovine serum albumin and 19 naturally occuring amino acids (20 µg/ml), leucine excluded, adjusted with 95% O_2:5% CO_2 at pH 7.4 for 2 h at 37 °C. (Pro)-insulin biosynthesis was estimated from the amount of radioactivity incorporated into the proinsulin/insulin fractions after ultrasonic disintegration of islets and TCA (final concentration 6.6%) precipitation respectively. The precipitated islet proteins were separated by gel filtration on a column of sephadex G 50/75 as previously published in detail[9]. Furthermore, aliquots of islet homogenates as well as incubation medium were taken for radioimmunological determinations of the insulin content and secretion of the islets[10].

Results and discussion. Our experiments show that incubation of mouse islets with glibenclamide for 48 h provoked a significant stimulation of insulin release but a suppression of insulin secretion in a subsequent short-term incubation (2 h) as well as in a longer culture period (48 h) even if the drug is no longer present (figure, table). For the cultivation period a stimulating glucose concentration (10 mmoles/l)



Insulin content and insulin release of cultured mice islets. 1st 48 h period: control=(10 mmoles/l glucose); test=glibenclamide (5 µg/ml) at 10 mmoles/l glucose. 2nd 48 h period (96 h): test and control H. 10 mmoles/l glucose.

Insulin secretion and ^{3}H-leucine incorporation into proinsulin/insulin fractions in short-time incubations (2 h) after exposure of islets to 10 mmoles/l glucose without glibenclamide (control) or with 5 µg/ml glibenclamide (test) for 48 h

		Insulin release (ng per islet in 2 h)	^{3}H-leucine incorporation (cpm per islet in 2 h)
Glucose			
(10 mmoles/l)	Control	2.4±0.3	1236±163.1
	Test	1.2±0.2*	1212± 74.2
Glucose			
(20 mmoles/l)	Control	2.8±0.2	1356±124.5
	Test	1.2±0.2*	1256± 76.6

Mean±SEM; n=12. * $p<0.01$ compared with controls.

was chosen, not a maximal one, because of the known ineffectiveness of sulfonylureas in potentiating insulin secretion at maximal glucose concentrations[11–13].

This inhibitory effect after exposure to glibenclamide on insulin release has recently been found by Borg[13], after cultivation of isolated islets from albino mice for 7 days at 5.5 mmoles/l glucose and glibenclamide, but not by Schatz et al.[7], who reported a stimulating effect after glibenclamide exposure. But a direct comparison with the latter work is not possible because the investigations of these authors were performed 1. with rat islets and 2. the incubation period in the presence of glibenclamide was considerably shorter (2 h).

Our results regarding the β-cell secretion hypofunction after exposure to glibenclamide are also in accordance with data described for tolbutamide and glibenclamide treatment in vivo,[4,5,14]. The low insulin content per islet (13 ng per islet) is not the only reason for the diminished insulin release, since also at unaltered insulin content (treatment with sulfonylureas in vivo) this secretory hypofunction was observed[4,5].

Pancreatic islets contain 50% of insulin only after culture with glibenclamide, compared with untreated controls (figure). Nevertheless, the incorporation rate of ^{3}H-leucine into (pro)insulin does not differ from controls and glibenclamide treated islets (table). Whether the presence of glibenclamide for 48 h provoked a reduction of insulin biosynthesis as described in short-term experiments[5,15,16] cannot be clearly answered at the moment. It is a fact, however, that the loss of stored insulin due to the stimulating effect of glibenclamide on hormone secretion is not compensated for by insulin biosynthesis (figure). The insulin stores are first filled up in the following 48 h period (without glibenclamide), since the insulin biosynthesis is glucose sensitive during diminished insulin release (figure). Our findings suggest that glibenclamide enhances the insulin secretion during long-time incubation in culture as described for short-time experiments too. The glucose-induced insulin release, however, is diminished after exposure to glibenclamide whereas the effectiveness of glucose on insulin biosynthesis is not restricted, i.e. glibenclamide pretreatment provoked a dissociation of glucose-responsiveness of insulin biosynthesis and release. This impairment of glucose-induced insulin release is reversible by cultivation with high glucose and is partly prevented if the glucose concentration is enhanced up to 20 mmoles/l glucose during the glibenclamide loading (unpublished data).

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Haemolymph ecdysteroids level following the injection of ecdysone or ecdysterone; its relation with tegument and midgut response in *Aeshna cyanea* (Insecta, Odonata)

J.C. Andries, P. Porcheron and F. Dray

Laboratoire de Biologie Animale et Laboratoire Associé au C.N.R.S., No. 148: 'Endocrinologie des Invertébrés', Université des Sciences et Techniques de Lille, F-59655 Villeneuve d'Ascq Cedex (France), Equipe de Cytophysiologie des Arthropodes, Equipe de Recherche Associée au C.N.R.S. No. 620, Université Pierre et Marie Curie, 105, Boulevard Raspail, F-75006 Paris, and Unité de Radio-Immunologie analytique, FRA I.N.S.E.R.M. 28, rue du Docteur Roux, F-75015 Paris (France), 16 July 1979

Summary. The haemolymph ecdysteroid level after injection of ecdysone or ecdysterone in *Aeshna cyanea* larvae has been determined by a radioimmunoassay method. The rate of excretion appears to be dependent on both the ecdysteroid injected and the time of injection. In case of ecdysone injection, the secretion of the epidermis cuticle and the differentiation of the imaginal midgut epithelium occur when the ecdysteroid level remains low for many days.

In an attempt to analyse the control of post-embryonic events in insects some investigators[1-4] have recently tried, by means of an elegant quantification of moulting hormone, to relate the amount of ecdysteroids to developmental events.

In a same way, in larvae of *Aeshna cyanea* injected with ecdysone or ecdysterone we have first determined from day to day the variations of the haemolymph ecdysteroids level, and then tried to correlate these levels to the response of both the epidermis and the midgut. It was also expected that this study would provide some information about the rates of excretion of exogenous ecdysteroids.

Material and methods. 116 last larval instars of *A. cyanea* were injected on day 1 or 5 with 20 µg of ecdysone (Simes)