Modulation of rat pancreatic islet cell replication and insulin release by glibenclamide

M.S. Khatim, H.A. Al-Mahmood and K.A. Gumaa

Department of Biochemistry, Faculty of Medicine, Kuwait University, Kuwait

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Glibenclamide significantly stimulated the incorporation of [^{3}H]thymidine into DNA of fetal rat pancreatic islets at a physiological (5.5 mM) but not at a high (22 mM) glucose concentration. There was no significant stimulation of insulin release under these conditions. In contrast, the drug-stimulated insulin release from adult islets cultured at 5.5 mM glucose but had no effect on their DNA synthesis. The observations suggest that insulin secretion and DNA synthesis may be dissociated in rat pancreatic islets.

Rat pancreatic islet *β-Cell replication* *Insulin release* *Glibenclamide*

1. INTRODUCTION

The stimulation of insulin release by pancreatic β-cells in response to sulphonylureas is well documented in vivo [1] and in vitro [2]. However, the precise mechanism behind this stimulation remains unclear. Evidence suggests that these compounds stimulate insulin release by mechanisms not involving increased metabolic fluxes [3]. There is also no agreement in the literature concerning the effects of sulfonylureas on the growth of pancreatic β-cells. Previous observations in vitro suggested that tolbutamide treatment of monolayer cultures of newborn rat pancreas caused a 3 to 4-fold increase in β-cell replication [4], though not in the isolated pancreatic islets of mice [5].

This study attempts to shed more light on the long-term actions of a hypoglycaemic sulfonylurea glibenclamide, with particular attention to the rate of DNA synthesis and insulin release in the β-cell.

2. MATERIALS AND METHODS

2.1. *Tissue culture*

Virgin female Wistar rats (200–250 g) were caged with males and sacrificed 21.5 days after confirmed mating. In this strain the duration of pregnancy is 22 days. Fetuses were immediately obtained by hysterotomy. Cultures of the fetal pancreas were prepared as in [6] and incubated at 37°C for 5 days in a humidified atmosphere of 5% CO_2 in air. The culture medium RPMI 1640 (Gibco), supplemented with 10% fetal calf serum was changed daily, and after 5 days the islets were transferred to fresh medium using a braking pipette [6].

medium supplemented with 5.5 or 22 mM glucose with or without the addition of glibenclamide (HB419, Hoechst). Islets from adult rat pancreas were obtained by a standard collagenase treatment [7] and tissue culture was performed at 5.5 or 11 mM glucose concentrations.

2.2. *Insulin release*

Groups of 20 fetal or 10 adult islets were transferred to multiwell tissue culture plates (Linbro) containing 200 μl Krebs-Ringer bicarbonate buffer (pH 7.4) supplemented with 5.5 mM glucose and 5 μM glibenclamide. Incubation was for a period of 90 min at 37°C in a humidified atmosphere of 5% CO_2 in air. Subsequently, 100 μl aliquots of the incubation medium were withdrawn and stored at -80°C until assayed for insulin as in [8].

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2.3. *Islet DNA synthesis*

Groups of islets were transferred to dishes containing 5 ml of the appropriate culture medium and 1 μCi/ml [*methyl*-^{3}H]thymidine (spec. act. 5 Ci/mmol, Radiochemical Centre, Amersham) and maintained in culture for a further 24 h. Islets were then washed in Hanks' balanced salt solution (HBSS) containing 10 mM unlabelled thymidine (Sigma) and once more in HBSS alone. Washed islets were transferred to plastic tubes containing 250 μl distilled water and disrupted ultrasonically. Aliquots (150 μl) of the homogenates were filtered through glass microfilters (Whatman) and the free [^{3}H]thymidine washed off the discs with distilled water. The filter discs were then dried overnight at room temperature and the radioactivity retained was then determined by liquid scintillation spectrometry. Duplicate aliquots of the homogenates (50 μl) were taken for the estimation of DNA [9] and the incorporation of [^{3}H]thymidine was expressed as dpm/μg DNA.

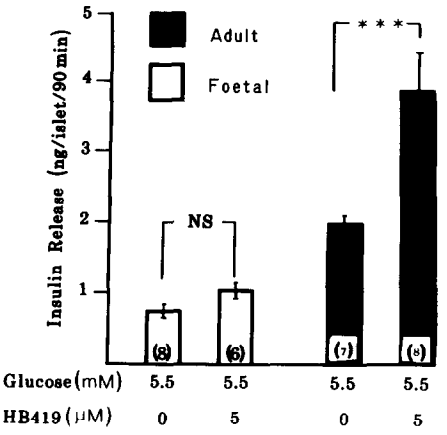

Fig.1. Effect of glibenclamide (HB419) on insulin release from isolated fetal and adult rat pancreatic islets. Insulin release was measured during a 90-min incubation period. Bars indicate means ± SE of the number of observations given in parentheses. Probability of chance differences between control, and treated islets. NS, $P > 0.05$; *** $P < 0.001$.

3. RESULTS

3.1. *Insulin release*

Fig.1 reveals that glibenclamide (5 μM) had no significant stimulatory effect on insulin release from fetal islets cultured in 5.5 mM glucose. In contrast, under identical conditions, adult islets responded with a significant increase in insulin release.

3.2. *DNA synthesis*

Table 1 shows that glibenclamide at 5 μM was without significant stimulatory effect on DNA synthesis in adult islets cultured at 5.5 mM glucose. Similarly, at concentrations of 2 and 10 μM, it had no effect when the culture medium contained 11 mM glucose. When fetal islets were treated with glibenclamide at 5 μM, there was a significant increase in DNA synthesis when these islets were

Table 1

Effects of glucose and glibenclamide on DNA synthesis in cultured rat pancreatis islets

Additions to culture	DNA synthesis (dpm/μg DNA)[a]	
	Adult	Fetal
Glucose (5.5 mM)	493 ± 53 (5)	1710 ± 179 (6)
+ glibenclamide (5 μM)	650 ± 51 (5) NS	4364 ± 807 (7)[b]
Glucose (11 mM)	696 ± 76 (28)	–
+ glibenclamide (2 μM)	696 ± 73 (21) NS	–
+ glibenclamide (10 μM)	659 ± 180 (8) NS	–
Glucose (22 mM)	–	2467 ± 248 (10)
+ glibenclamide (5 μM)	–	2864 ± 472 (8) NS

[a] Results are given as the means of the numbers of observations given in parentheses ± SE. Probability of the chance difference between control, and glibenclamide treated islets: NS, $P > 0.05$

[b] $P < 0.05$

cultured at 5.5 but not at 22 mM glucose. Moreover, fetal islets incorporated more [^{3}H]thymidine than adult islets cultured under similar conditions ($P < 0.001$). Note also that the increase in the glucose concentration had a significant stimulatory effect on the incorporation of [^{3}H]thymidine into fetal and adult islet DNA.

4. DISCUSSION

The observed increase in insulin release from adult rat islets in response to glibenclamide confirms that this sulfonylurea retains its insulin-releasing properties under the present in vitro conditions. Failure of fetal islets to respond, with increased insulin release, to glibenclamide in vitro may reflect the immature state of their β-cells which are known to have a low insulin response to glucose after being maintained for a week at 5.6 mM glucose [10]. In [4], tolbutamide was found to enhance insulin release from monolayer cultures of neonatal rat pancreas, but the degree of enhancement (25%) was not statistically significant as reported here.

Failure of glibenclamide to enhance DNA replication in vitro in adult rat islets cultured at either 5.5 or 11 mM glucose was not unexpected, in agreement with the reported inability of tolbutamide to stimulate DNA replication in adult mouse islets maintained at a physiological glucose concentration [5]. In contrast, fetal islets were found to respond to glibenclamide with an enhancement of DNA synthesis when the glucose concentration in the culture medium was in the physiological range. Together with data in [4], the present observations indicate that in the fetal β-cell, both glibenclamide and tolbutamide are potent stimulators of DNA replication in vitro, at least at physiological glucose concentrations. It should be noted that the incorporation of [^{3}H]-thymidine into DNA may not accurately reflect DNA replication if the precursor pool was altered, such as changes in its uptake or phosphorylation in these cells [11].

Our observations suggest furthermore, that insulin release and DNA replication are apparently dissociated functions when β-cells are exposed to sulphonylureas in support of the conclusion in [12]. This is in contrast with glucose, which stimulated both functions in concert, acting as a nutrient to the β-cell [6]. The differences between glucose and the sulfonylureas may reflect that the effects of the latter could be mediated via mechanisms other than nutrient receptors in the β-cells [3], and indicate that functional stimulation of these cells is not a prerequisite for increased replication.

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