ELSEVIER

European Journal of Pharmacology 408 (2000) 327–333

ejp

www.elsevier.nl/locate/ejphar

Desensitization of sulphonylurea- and nutrient-induced insulin secretion following prolonged treatment with glibenclamide

Andrew J. Ball, Peter R. Flatt, Neville H. McClenaghan *

School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, UK

Received 27 April 2000; received in revised form 29 September 2000; accepted 11 October 2000

Abstract

Functional effects of prolonged exposure to the sulphonylurea glibenclamide were examined in a popular clonal pancreatic β-cell line, denoted as BRIN-BD11. In acute 20-min incubations, 200 μM of tolbutamide or glibenclamide stimulated insulin release from non-depolarized and depolarized cells, which was dramatically reduced following 18-h culture with 100 μM glibenclamide. Sulphonylurea desensitization in non-depolarized cells was reversed following 6–36-h subsequent culture in the absence of glibenclamide. However, desensitization of insulinotropic effects of sulphonylureas in depolarized cells following glibenclamide culture and associated decline in cellular insulin content was not fully reversible. Culture with 100 μM glibenclamide also markedly reduced the acute insulinotropic actions of glucose, L-alanine, L-arginine, 2-ketoisocaproic acid (KIC) and KCl. These effects were almost completely reversed following 18-h culture in the absence of the sulphonylurea.

Keywords: Glibenclamide; Tolbutamide; Desensitization; Clonal pancreatic β-cell; Insulin release; K_{ATP} channel

1. Introduction

The insulinotropic sulphonylurea glibenclamide is well established as a therapeutic tool for the treatment of type 2 diabetes (non-insulin-dependent diabetes mellitus, NIDDM) (Groop, 1997). Like other sulphonylureas, glibenclamide increases insulin secretion by directly closing ATP-sensitive K^+ (K_{ATP}) channels in pancreatic β-cells (Cook et al., 1998), causing membrane depolarization, opening of voltage-dependent Ca^{2+} channels, leading to influx and elevation of intracellular Ca^{2+}, triggering exocytosis of insulin (Hellman et al., 1994). The primary actions of glibenclamide on K_{ATP} channels are exerted through binding to a high affinity site, localized on the SUR1 K_{ATP} channel subunit (Aguilar-Bryan and Bryan, 1999; Ashfield et al., 1999). A recent study has also demonstrated that the Kir6.2 K_{ATP} channel subunit possesses a low affinity binding site for glibenclamide (Gros et al., 1999); however, the clinical relevance remains unknown.

Unlike most other sulphonylureas, glibenclamide is known to be internalized within the pancreatic β-cell (Hellman et al., 1984; Carpentier et al., 1986; Marynissen et al., 1992), thus suggesting that the insulinotropic action of glibenclamide may not be solely ascribed to events at the cell surface (Carpentier et al., 1986). Subsequent studies indicate that sulphonylureas have a direct effect on exocytosis (Flatt et al., 1994), and that glibenclamide and tolbutamide influence insulin secretion independently of K_{ATP} channel closure (Eliasson et al., 1996; Fuhlendorff et al., 1998; Efanov et al., 1998). Indeed, the majority of sulphonylurea receptors (SUR1) are bound to intracellular membranes (Ozanne et al., 1995), and it is therefore perhaps not surprising that glibenclamide may influence insulin exocytosis by exerting intracellular β-cell actions.

Although the sulphonylurea drugs are widely adopted in long-term type 2 diabetes therapy (DeFronzo, 1998), relatively little attention has been paid to the reported progressive β-cell sulphonylurea insensitivity associated with long-term exposure. A growing body of evidence has accumulated over the past 30 years, suggesting that the decline in glucose-lowering ability during long-term exposure to sulphonylureas both in vitro and in vivo may be attributable to a desensitization of the β-cell to the actions of these drugs (Sodoyez et al., 1970; Dunbar and Foa,

* Corresponding author. Tel.: +44-28-7032-3011; fax: +44-28-7032-4965.

E-mail address: nh.mcclenaghan@ulst.ac.uk (N.H. McClenaghan).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00782-2

1974; Borg and Andersson, 1980; Filiponni et al., 1983; Karam et al., 1986; Rabuazzo et al., 1992; Grunberger, 1993).

Long-term in vitro studies using isolated pancreatic islets are invariably hindered by a number of inherent functional inadequacies, particularly the decline in insulin production and islet cell integrity with time in tissue culture (Halban and Wollheim, 1980). The pancreatic BRIN-BD11 cell line, derived from rat β-cell-RINm5F cell electrofusion (hence the name BRIN), is both phenotypically and functionally stable in culture, and responsive to a wide range of modulators of insulin secretion, including sulphonylureas (McClenaghan et al., 1996a; McClenaghan and Flatt, 1999a,b; Salgado et al., 1999). BRIN-BD11 cells have been successfully adopted in studies examining the effects of prolonged exposure to tolbutamide, efaroxan and the novel insulinotropic drug 1,1-dimethyl-2-(2-morpholinophenyl)guanidine fumarate, BTS 67 582 (Ball et al., 1999; Chapman et al., 1999; McClenaghan et al., 1999). The present study utilizes the BRIN-BD11 cell line to provide an assessment of the effects of prolonged treatment with the popular antidiabetic agent, glibenclamide, on the functions and responsiveness of insulin secreting cells.

2. Materials and methods

2.1. Chemicals

Reagents of analytical grade and deionised water (Purite, Oxon, UK) were used. RPMI-1640 tissue culture medium, foetal bovine serum, antibiotics, Hanks' balanced saline solution (HBSS) and trypsin/EDTA were from Life Technologies (Paisley, UK). Rat insulin standard was from Novo-Nordisk (Bagsvaerd, Denmark) and [^{125}I]-bovine insulin for radioimmunoassay was from Lifescreen (Watford, UK). All other chemicals including glibenclamide and tolbutamide were from Sigma or BDH Chemicals (both of Poole, Dorset, UK).

2.2. Cell culture and measurement of insulin release and cellular insulin content

Clonal pancreatic BRIN-BD11 cells (passage numbers 20–30) were used for this study (McClenaghan et al., 1996a). BRIN-BD11 cells were grown in RPMI-1640 tissue culture medium containing 11.1 mmol/l glucose and 0.3 g/l L-glutamine, and supplemented with 10% (v/v) fetal calf serum, 100 IU/ml penicillin and 0.1 g/l streptomycin at 37°C with 5% CO_2 and 95% air. Cells were washed with HBSS prior to detachment from tissue culture flasks with the aid of 0.025% trypsin containing 1 mM EDTA, and seeded at 1.5×10^5 cells/well into 24-multiwell plates. Monolayers of cells were then cultured for 18 h at 37°C. Culture medium was then replaced with 1 ml of a Krebs Ringer Bicarbonate (KRB) buffer (pH 7.4) supplemented with 0.1% bovine serum albumin and 1.1 mmol/l glucose (McClenaghan et al., 1996a). After 40 min preincubation at 37°C, the buffer was replaced with 1 ml of KRB test buffer containing glucose and test agents as detailed in the legends to figures. After 20 min incubation at 37°C, aliquots of test buffer were removed and stored at −20°C for insulin radioimmunoassay (Flatt and Bailey, 1981). Cellular insulin content was similarly measured following overnight extraction with acid–ethanol solution (1.5% (v/v) HCl, 75% (v/v) ethanol, 23.5% (v/v) H_2O) added (McClenaghan et al., 1996a).

2.3. Statistical analysis

Results are presented as mean ± S.E.M. for a given number of observations. Groups of data were compared using unpaired Student's *t*-test and two-way ANOVA in conjunction with Scheffe's and Bonferroni's modified *t*-statistics. Differences were considered significant if $P < 0.05$.

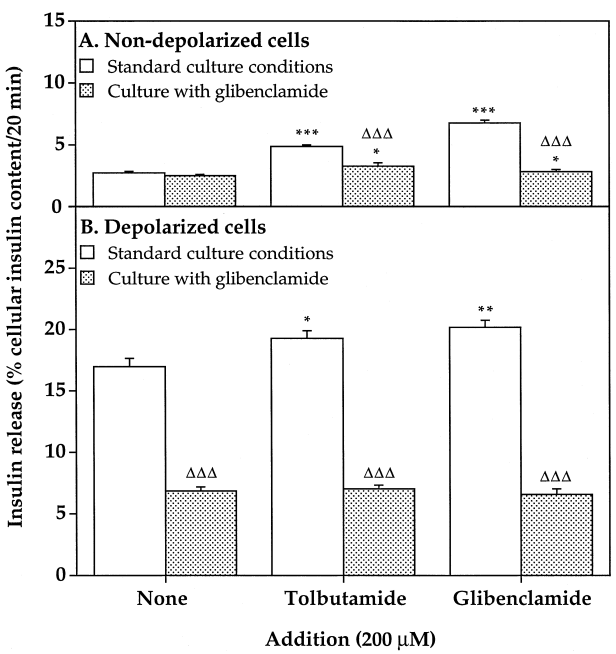

Fig. 1. Effects of prolonged exposure to glibenclamide or tolbutamide upon sulphonylurea-induced insulin secretion from non-depolarized (A) and depolarized (B) cells. BRIN-BD11 cells were cultured for 18 h in either standard tissue culture medium (standard culture conditions) or in tissue culture medium supplemented with 100 μM glibenclamide. Following 40 min preincubation at 1.1 mM glucose, effects of 200 μM tolbutamide or 200 μM glibenclamide were tested during 20 min incubation in the presence of 1.1 mM glucose (A), or 16.7 mM glucose plus 30 mM KCl (B). Each column represents the mean ± S.E.M. of six separate observations. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with effect in absence of 200 μM tolbutamide or glibenclamide. $^{\Delta\Delta\Delta}P < 0.001$ compared with respective effects after standard culture conditions.

3. Results

3.1. Desensitization of K_{ATP} channel-dependent and -independent sulphonylurea-induced insulin secretion following prolonged glibenclamide treatment

Following 18-h exposure to standard culture conditions, 200 μM tolbutamide (1.8-fold increase; $P < 0.001$) or 200 μM of glibenclamide (2.5-fold increase; $P < 0.001$) elicited characteristic insulinotropic responses at 1.1 mM glucose (Fig. 1A). However, 18-h culture with 100 μM glibenclamide caused a dramatic reduction the subsequent insulin releasing action of both sulphonylurea drugs. While the acute insulinotropic effect of tolbutamide was decreased by 64% ($P < 0.001$), the stimulatory action of glibenclamide at 1.1 mM glucose was reduced by 92% ($P < 0.001$) corresponding to 33% and 58% decreases in insulin output, respectively (Fig. 1A).

In cells exposed to conditions of marked membrane depolarization (16.7 mM glucose plus 30 mM KCl), both tolbutamide and glibenclamide significantly augmented ($P < 0.05$–$P < 0.01$) insulin-secretory output (Fig. 1B). Culture for 18 h in the presence of 100 μM glibenclamide exerted a profound inhibitory effect upon subsequent insulin release from depolarized cells (60–67% decrease; $P < 0.001$) abolishing the acute stimulatory effects of tolbutamide and glibenclamide.

The nature of induced desensitization of sulphonylurea-induced insulin secretion was further assessed by examining cells cultured for 18 h at different glibenclamide concentrations (Fig. 2). Both tolbutamide (1.4-fold increase; $P < 0.01$) and glibenclamide (2.1-fold increase; $P < 0.001$) stimulated insulin secretion in the presence of 1.1 mM glucose following 18-h standard culture (Fig. 2). Culture for 18 h at 1–25 μM glibenclamide did not affect subsequent insulin-secretory responses to either sulphonylurea (Fig. 2). However, exposure to 50–200 μM glibenclamide resulted in a significant concentration-dependent decrease in insulin output in the absence (17–75% decrease; $P < 0.05$–$P < 0.001$) or presence of 200 μM of tolbutamide (23–82% decrease; $P < 0.01$–$P < 0.001$) or glibenclamide (38–87%; $P < 0.001$) (Fig. 2). Cell viability assessed by trypan blue exclusion, the neutral red assay and MTT assay (Hunt et al., 1987; Janjic and Wollheim, 1992; McClenaghan et al., 1996a) was not changed by any of the experimental manipulations (data not shown).

3.2. Reversibility of desensitization of sulphonylurea-induced insulin secretion

Reversibility of the desensitizing effects of 100 μM glibenclamide culture on insulin release was assessed following subsequent return to standard culture conditions for 0–36 h. Since 18-h culture with 100 μM glibenclamide caused an irreversible 34% ($P < 0.001$) decrease in cellular insulin content compared with standard culture conditions (71.2 ± 2.4 ng/10^6 cells; mean ± S.E.M., $n = 18$), all subsequent data were expressed as a percentage of

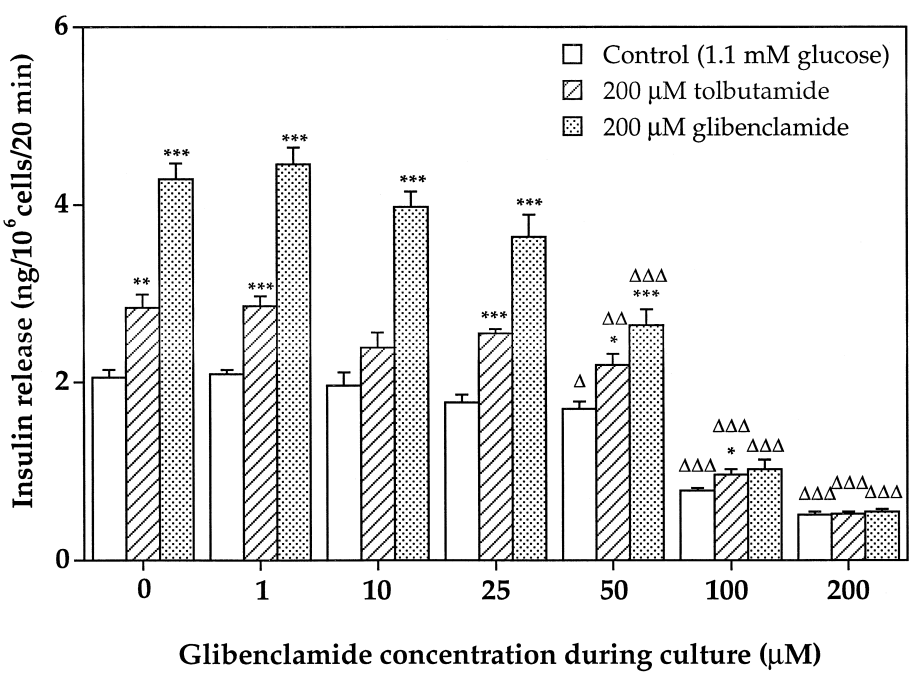

Fig. 2. Effects of 18 h culture with a range of glibenclamide concentrations on subsequent acute responsiveness to tolbutamide or glibenclamide. BRIN-BD11 cells were cultured for 18 h in either standard tissue culture medium, or in tissue culture medium supplemented with 1–200 μM glibenclamide. Following 40 min preincubation at 1.1 mM glucose, effects of 200 μM tolbutamide or 200 μM glibenclamide were tested during a 20-min incubation. Each column represents the mean ± S.E.M. of six separate observations. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with effect in absence of 200 μM tolbutamide or glibenclamide. $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, $^{\Delta\Delta\Delta}P < 0.001$ compared with effect in absence of glibenclamide during 18 h culture.

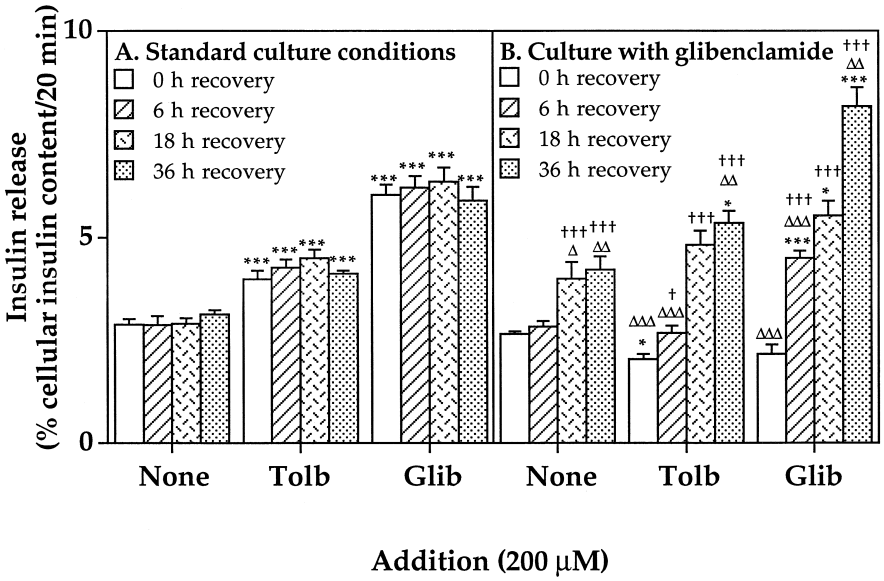

Fig. 3. Effects of recovery time on responsiveness to tolbutamide and glibenclamide in cells previously exposed to glibenclamide in culture. BRIN-BD11 cells were initially cultured for 18 h under standard culture conditions (A), or in medium supplemented with 100 μM glibenclamide (B). Media were removed after 18 h and cells were cultured for a further 6, 18, or 36 h (recovery time) in standard culture medium. Following 40 min preincubation at 1.1 mM glucose, effects of 200 μM tolbutamide or 200 μM glibenclamide were tested during a 20-min incubation period. Each column represents the mean ± S.E.M. of six separate observations. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with effect in absence of 200 μM tolbutamide or glibenclamide. $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, $^{\Delta\Delta\Delta}P < 0.001$ compared with respective effects after standard culture conditions. $^{\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.001$ compared with 0 h recovery.

cellular insulin content, thus allowing direct comparison between conditions.

Consistent with the good functional stability, insulin secretion from BRIN-BD11 cells was unaffected by further 6, 18 or 36 h exposure to standard culture (Fig. 3A). However, in cells previously exposed to 100 μM glibenclamide for 18 h, basal insulin secretion (1.1 mM glucose) was progressively increased by 57–59% ($P < 0.001$) after

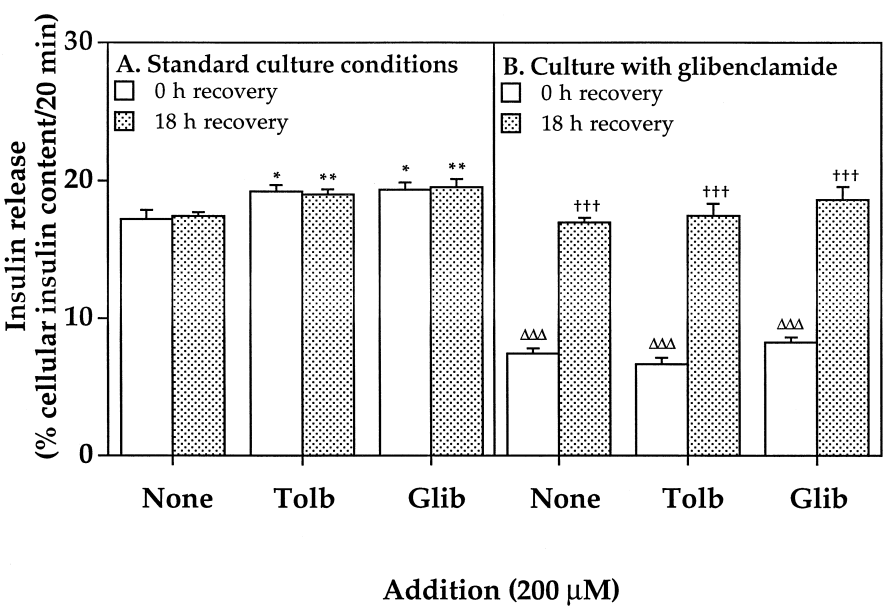

Fig. 4. Effects of 18 h recovery time on responsiveness to tolbutamide and glibenclamide in depolarized cells previously exposed to glibenclamide in culture. BRIN-BD11 cells were initially cultured for 18 h under standard culture conditions (A), or in medium supplemented with 100 μM glibenclamide (B). Media were removed after 18 h and cells were cultured for a further 18 h (recovery time) in standard culture medium. Following 40 min preincubation at 1.1 mM glucose, effects of 200 μM tolbutamide or 200 μM glibenclamide were tested during 20 min incubation in the presence of 16.7 mM glucose plus 30 mM KCl. Each column represents the mean ± S.E.M. of six separate observations. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with effect in absence of 200 μM tolbutamide or glibenclamide. $^{\Delta\Delta\Delta}P < 0.001$ compared with respective effects after standard culture conditions. $^{\dagger\dagger\dagger}P < 0.001$ compared with 0 h recovery.

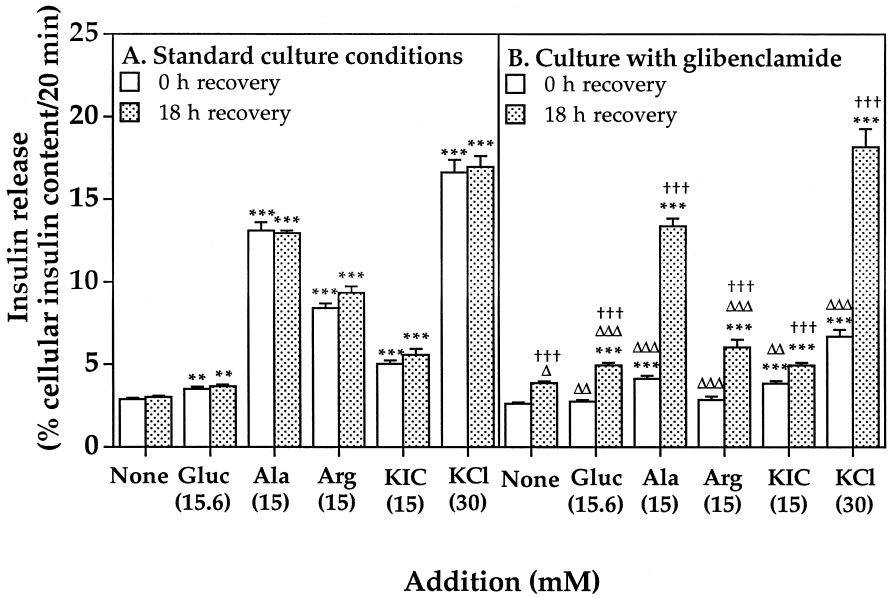

Fig. 5. Effects of 18 h recovery time on responsiveness to glucose, L-alanine, L-arginine, 2-ketoisocaproate and KCl in cells previously exposed to glibenclamide in culture. BRIN-BD11 cells were initially cultured for 18 h under standard culture conditions (A), or in medium supplemented with 100 μM glibenclamide (B). Media were then removed after 18 h and cells were cultured for a further 18 h (recovery time) in standard culture medium. Following 40 min preincubation at 1.1 mM glucose, effects of 16.7 mM glucose, 15 mM L-alanine (Ala), 15 mM L-arginine (Arg), 15 mM 2-ketoisocaproate (KIC) or 30 mM KCl were tested during 20 min incubation. Each column represents the mean ± S.E.M. of six separate observations. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with effect in absence of addition. $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, $^{\Delta\Delta\Delta}P < 0.001$ compared with respective effects after standard culture conditions. $^{\dagger\dagger\dagger}P < 0.001$ compared with 0 h recovery.

18–36 h recovery in standard culture (Fig. 3B). A similar pattern emerged for acute insulin secretory responses to 200 μM tolbutamide (31%, 138% and 164% responses, $P < 0.05$–$P < 0.001$) and 200 μM glibenclamide (108%, 156% and 278%, $P < 0.001$) after 6, 18 and 36 h recovery, respectively (Fig. 3B). Indeed, full secretory responsiveness to both sulphonylureas was re-established by 18–36 h after glibenclamide culture.

To assess the effects of glibenclamide culture on the K_{ATP} channel-independent actions of sulphonylureas, acute insulin secretory responses to tolbutamide and glibenclamide were examined in the presence of 16.7 mM glucose plus 30 mM KCl. As shown in Fig. 4A, the K_{ATP} channel-independent insulinotropic actions of tolbutamide and glibenclamide were not altered by a further 18-h culture under standard conditions. Consistent with Fig. 3B, the reduction in basal insulin output following culture with glibenclamide was largely reversed by 18-h subsequent exposure to standard culture conditions (Fig. 4B). Similarly, as shown in Fig. 4B, the K_{ATP} channel-independent effects of glibenclamide and tolbutamide were almost completely restored by 18-h post-glibenclamide culture.

3.3. Effects of glibenclamide culture on the actions of other insulin secretagogues

Characteristic of BRIN-BD11 cells cultured under standard conditions (McClenaghan et al., 1996a,b; McClenaghan and Flatt, 1999c, 2000), glucose, L-alanine, L-arginine, 2-ketoisocaproic acid (KIC) and KCl induced respective 1.2-, 4.6-, 2.9-, 1.7- and 5.8-fold ($P < 0.01$–$P < 0.001$) insulin secretory responses (Fig. 5A). None of these responses were affected by a further 18-h culture under standard conditions. In contrast, 18-h culture with 100 μM glibenclamide significantly reduced insulin output ($P < 0.01$–$P < 0.001$) in response to each secretagogue tested. Whereas insulin output in response to L-alanine, L-arginine and KCl was dramatically decreased by 68%, 66% and 60% ($P < 0.001$), the effects of glucose and KIC were only modestly reduced (22% and 23%, $P < 0.01$) (Fig. 5B). Interestingly, an 18-h period of recovery in standard culture conditions was sufficient to restore the insulinotropic responses to glucose, L-alanine, KIC and KCl, while arginine despite showing significantly enhanced ($P < 0.001$) insulinotropic activity, did not achieve the secretory potency observed under normal culture conditions (Fig. 5B).

4. Discussion

The present study demonstrates that prolonged exposure of insulin-secreting cells to glibenclamide results in desensitization of the insulinotropic actions of a variety of secretagogues. In this study, particular emphasis was directed to examining the functional consequences of prolonged glibenclamide exposure on responsiveness to

sulphonylureas and other important regulators of K_{ATP} channel activity and membrane depolarization.

Consistent with previous observations, both glibenclamide and tolbutamide initiated insulin secretory responses at non-stimulatory (1.1 mM) glucose (McClenaghan et al., 1999), which were desensitized after 18-h culture with glibenclamide. Notably, the K_{ATP} channel-independent effects of these drugs (Flatt et al., 1994; Eliasson et al., 1996; Efanov et al., 1998; Tian et al., 1998; McClenaghan et al., 1999) were also removed after glibenclamide culture, supporting the hypothesis that prolonged exposure to sulphonylureas desensitizes pancreatic β-cells in type 2 diabetes (Dunbar and Foa, 1974; Filiponni et al., 1983; Karam et al., 1986; Rabuazzo et al., 1992; Grunberger, 1993). In addition to extending the desensitization phenomenon previously reported for tolbutamide, BTS 67 582 and efaroxan (Ball et al., 1999; Chapman et al., 1999; McClenaghan et al., 1999; 2000), the present data indicate that glibenclamide-induced desensitization reflects a change in both K_{ATP} channel-dependent and K_{ATP} channel-independent insulinotropic pathways (Nelson et al., 1992; Flatt et al., 1994; Eliasson et al., 1996; McClenaghan et al., 1999).

The glibenclamide-induced desensitization followed a progressive concentration-dependent pattern, with maximal effects at 100–200 μM and the decline in basal and sulphonylurea-induced insulin release was associated with a 34% decrease in cellular insulin content. This corresponds with the known inability of glibenclamide to promote insulin biosynthesis (Grodsky et al., 1977; Schatz et al., 1977, 1978). Interestingly, this negative effect on insulin content was not induced by prolonged culture with tolbutamide, or indeed BTS 67 582 or efaroxan (Ball et al., 1999; Chapman et al., 1999; McClenaghan et al., 1999, 2000), reflecting differences in the modes of action of these insulinotropic drugs. Further studies are required to assess whether this is related to the unique ability of glibenclamide to be internalized and bind to secretory granules within the β-cells (Hellman et al., 1984; Carpentier et al., 1986; Marynissen et al., 1992; Flatt et al., 1994; Ozanne et al., 1995).

Further studies examined the reversibility of the glibenclamide-induced desensitization, and to distinguish insulin secretory from biosynthetic steps, insulin release data was expressed as a percentage of total insulin content. Interestingly, the detrimental effects induced by 18-h exposure to glibenclamide showed a time-dependent recovery with a complete restoration of sulphonylurea-induced insulin release by 18–36 h. However, reversal of K_{ATP} channel-independent actions of sulphonylureas were less readily restored after 18-h exposure to glibenclamide, compared with tolbutamide culture (McClenaghan et al., 1999), perhaps reflecting slower wash-out of glibenclamide from intracellular binding sites.

Additional experiments examined the effects of glibenclamide culture on the actions of agents primarily exerting their secretory effect through modulation of membrane electrical activity and K_{ATP} channel function (McClenaghan et al., 1996a,b; McClenaghan and Flatt, 1999c, 2000). Glibenclamide culture effectively impaired the actions of glucose, L-alanine, L-arginine, KIC and a depolarizing concentration of KCl. Only the effect of L-arginine could not be fully restored after an 18-h recovery period. Extension of glibenclamide desensitization to alteration of the actions of these agents contrasts with effects of prolonged exposure to tolbutamide and efaroxan, which primarily impair drug-induced insulin release (Chapman et al., 1999; McClenaghan et al., 1999). These differences may reflect an underlying toxic action related to internalization and intracellular activity of glibenclamide (Carpentier et al., 1986; Flatt et al., 1994; Ozanne et al., 1995; Eliasson et al., 1996; McClenaghan et al., 1999), which may include chronic elevation of intracellular Ca^{2+} concentrations (Efanova et al., 1998).

In conclusion, this study has demonstrated that prolonged exposure of insulin secreting cells to glibenclamide has a broad spectrum of inhibitory effects on important physiological and pharmacological insulinotropic pathways. These inhibitory effects are more profound than equimolar efaroxan, BTS 67 582 and tolbutamide, but reversibility of these actions argues against a purely toxic effect of glibenclamide.

Acknowledgements

These studies were supported in part by the Research and Development Office of the Northern Ireland Department of Health and Personal Social Services.

References

Aguilar-Bryan, L., Bryan, J., 1999. Molecular biology of adenosine triphosphate-sensitive potassium channels. Endocr. Rev. 20, 101–135.

Ashfield, R., Gribble, F.M., Ashcroft, S.J., Ashcroft, F.M., 1999. Identification of the high-affinity tolbutamide site on the SUR1 subunit of the K_{ATP} channel. Diabetes 48, 1341–1347.

Ball, A.J., McClenaghan, N.H., Flatt, P.R., 1999. BTS 67 582 has common and distinct beta cell actions to sulphonylurea and imidazoline drugs. Diabetologia 42 (Suppl. 1), A244.

Borg, H.L.A., Andersson, A., 1980. Long-term effects of glibenclamide on the insulin production, oxidative metabolism and quantitative ultrastructure of mouse pancreatic islets maintained in tissue culture at different glucose concentrations. Acta Diabetol. Lat. 18, 65–83.

Carpentier, J.-L., Sawano, F., Ravazzola, M., Malaisse, W.J., 1986. Internalization of ^{3}H-glibenclamide in pancreatic islet cells. Diabetologia 29, 259–261.

Chapman, J.C., McClenaghan, N.H., Cosgrove, K.E., Hasmi, M.N., Shepherd, R.M., Giesberts, A.N., White, S.J., Ammala, C., Flatt, P.R., Dunne, M.J., 1999. ATP-sensitive potassium channels and efaroxan-induced insulin release in the electrofusion-derived BRIN-BD11 beta cell line. Diabetes 48, 2349–2357.

Cook, D.L., Stain, L.S., Ashfold, M.L.J., Hales, C.N., 1998. ATP-sensitive K^+ channels in pancreatic β-cells. Diabetes 37, 495–498.

DeFronzo, R.A., 1998. Current Therapy of Diabetes Mellitus. Mosby, St. Louis.

Dunbar, J.C., Foa, P.P., 1974. An inhibitory effect of tolbutamide and glibenclamide (glyburide) on the pancreatic islets of normal animals. Diabetologia 10, 27–35.

Efanov, A.M., Zaitsev, S.V., Efanova, I.B., Zhu, S., Ostenson, C.G., Berggren, P.O., Efendic, S., 1998. Signaling and sites of interaction for RX-871024 and sulfonylurea in the stimulation of insulin release. Am. J. Physiol. 274, E751–E757.

Efanova, I.B., Zaitsev, S.V., Zhivotovsky, B., Kohler, M., Efendic, S., Orrenius, S., Berggren, P.O., 1998. Glucose and tolbutamide induce apoptosis in pancreatic beta-cells. A process dependent on intracellular Ca^{2+} concentration. J. Biol. Chem. 273, 33501–33507.

Eliasson, L., Renstrom, E., Ammala, C., Berggren, P.-O., Bertorello, A.M., Bokvist, K., Chibalin, A., Deeney, J.T., Flatt, P.R., Gabel, J., Gromada, J., Larsson, O., Lindström, P., Rhodes, C.J., Rorsman, P., 1996. PKC-dependent stimulation of exocytosis by sulfonylureas in pancreatic β-cells. Science 271, 813–815.

Filiponni, P., Marcelli, M., Nicoletti, I., Pacifici, R., Saneusanio, F., Brunetti, P., 1983. Suppressive effect of long-term sulphonylurea treatment on A, B, and D cells of normal rat pancreas. Endocrinology 113, 1972–1979.

Flatt, P.R., Bailey, C.J., 1981. Abnormal plasma glucose and insulin responses in heterozygous (ob/+) mice. Diabetologia 20, 573–577.

Flatt, P.R., Shibier, O., Szecowka, J., Berggren, P.-O., 1994. New perspectives on the actions of sulphonylureas and hyperglycamic sulphonamides on the pancreatic B-cell. Diabete Metab. 20, 157–162.

Fuhlendorff, J., Rorsman, P., Kofod, H., Brand, C.L., Rolin, B., MacKay, P., Shymko, R., Carr, R., 1998. Stimulation of insulin release by repaglinide and glibenclamide involves both common and distinct processes. Diabetes 47, 345–351.

Grodsky, G.M., Epstein, G.H., Fanska, R., Karam, J.H., 1977. Pancreatic actions of sulphonylureas. Fed. Proc. 36, 2714–2719.

Groop, L.C., 1997. Drug treatment of non-insulin-dependent diabetes mellitus. In: Pickup, J.C., Williams, G. (Eds.), Textbook of Diabetes. Blackwell Science, Oxford, pp. 38.1–38.18.

Gros, L., Virsolvy, A., Salazar, G., Bataille, D., Blache, P., 1999. Characterization of low-affinity binding sites for glibenclamide on the Kir6.2 subunit of the β-cell K_{ATP} channel. Biochem. Biophys. Res. Commun. 257, 766–770.

Grunberger, G., 1993. Continuous versus intermittent sulphonylurea therapy in non-insulin-dependent diabetes mellitus. Drug Saf. 9, 249–253.

Halban, P.A., Wollheim, C.B., 1980. Intracellular degradation of insulin stores by pancreatic islets in vitro: an alternative pathway for homeostasis of pancreatic insulin content. J. Biol. Chem. 255, 6003–6006.

Hellman, B., Sehlin, J., Täljedal, I.-B., 1984. Glibenclamide is exceptional among hypoglycaemic sulphonylureas in accumulating progressively in β-cell rich pancreatic islets. Acta Endocrinol. (Copenhagen) 105, 385–390.

Hellman, B., Gylfe, E., Bergsten, P., Grapengeisser, E., Lund, P.-E., Saha, S., Berts, A., Dryselius, S., Tengholm, A., Liu, Y.-J., Eberhardson, M., 1994. Pulsatile Ca^{2+} signalling and insulin release. In: Flatt, P.R., Lenzen, S. (Eds.), Frontiers of Insulin Secretion and Pancreatic B-Cell Research. Smith-Gordon, London, pp. 221–228.

Hunt, S.M., Chrzanowska, C., Barnett, C.R., Brand, H.N., Fawell, J.K., 1987. A comparison of in vitro cytotoxicity assays and their application to water samples. ATLA-Altern. Lab. Anim. 15, 20–29.

Janjic, D., Wollheim, C.B., 1992. Islet cell metabolism is reflected by the MTT (tetrazolium) colorimetric assay. Diabetologia 35, 482–485.

Karam, J.H., Sanz, E., Salmomon, E., Nolte, M.S., 1986. Selective unresponsiveness of pancreas B-cells to acute sulphonylurea stimulation during sulphonylurea therapy in NIDDM. Diabetes 35, 1314–1320.

Marynissen, G., Smets, G., Klöppel, G., Gerlache, L., Malaisse, W.J., 1992. Internalization of glimepiride and glibenclamide in the pancreatic B-cell. Acta Diabetologia 29, 113–114.

McClenaghan, N.H., Flatt, P.R., 1999a. Engineering cultured insulin-secreting pancreatic B-cell lines. J. Mol. Med. 77, 235–243.

McClenaghan, N.H., Flatt, P.R., 1999b. Physiological and pharmacological regulation of insulin release: insights offered through exploitation of insulin-secreting cell lines. Diabetes, Obes. Metab. 1, 137–150.

McClenaghan, N.H., Flatt, P.R., 1999c. Glucose and non-glucidic nutrients exert permissive effects on 2-keto acid regulation of pancreatic B-cell function. Biochim. Biophys. Acta 1426, 110–118.

McClenaghan, N.H., Flatt, P.R., 2000. Metabolic and K_{ATP} channel-independent actions of keto acid initiators of insulin secretion. Pancreas 20, 38–46.

McClenaghan, N.H., Barnett, C.R., Ah-Sing, E., Abdel-Wahab, Y.H., O'Harte, F.P., Yoon, T.W., Swanston-Flatt, S.K., Flatt, P.R., 1996a. Characterization of a novel glucose-responsive insulin-secreting cell line, BRIN-BD11, produced by electrofusion. Diabetes 45, 1132–1140.

McClenaghan, N.H., Barnett, C.R., O'Harte, F.P., Flatt, P.R., 1996b. Mechanisms of amino acid-induced insulin secretion from the glucose-responsive BRIN-BD11 pancreatic B-cell line. J. Endocrinol. 151, 349–357.

McClenaghan, N.H., Ball, A.J., Flatt, P.R., 1999. Downregulation of K_{ATP}-channel-dependent and -independent insulinotropic actions of tolbutamide. Diabetologia 42 (Suppl. 1), A130.

McClenaghan, N.H., Ball, A.J., Flatt, P.R., 2000. Induced desensitization of the insulinotropic effects of antidiabetic drugs BTS 67 582 and tolbutamide. Br. J. Pharmacol. 130, 478–484.

Nelson, D.A., Aguilar-Bryan, L., Raef, H., Boyd, A.E., 1992. Molecular mechanisms of sulphonylurea action in the pancreatic B-cell. In: Flatt, P.R. (Ed.), Nutrient Regulation of Insulin Secretion. Portland Press, London, pp. 319–339.

Ozanne, S.E., Guest, P.C., Hutton, J.C., Hales, C.N., 1995. Intracellular localization and molecular heterogeneity of the sulphonylurea receptor in insulin-secreting cells. Diabetologia 38, 277–282.

Rabuazzo, A.M., Buscema, M., Vinci, C., Caltabiano, V., Vetri, M., Forte, F., Vigneri, R., Purrello, F., 1992. Glyburide and tolbutamide induce desensitization of insulin release in rat pancreatic islets by different mechanisms. Endocrinology 131, 1815–1820.

Salgado, A.P., Pereira, F.C., Seica, R.M., Fernandes, A.P., Flatt, P.R., Santos, R.M., Rosario, L.M., Ramasamy, R., 1999. Modulation of glucose-induced insulin secretion by cytosolic redox state in clonal β-cells. Mol. Cell. Endocr. 154, 79–88.

Schatz, H., Steinle, D., Pfeiffer, E.F., 1977. Long-term actions of sulphonylureas on (pro-) insulin biosynthesis and secretion: I. Lack of evidence for a compulsory increase in (pro-) insulin biosynthesis after exposure of isolated pancreatic rat islets to tolbutamide and glibenclamide in vitro. Horm. Metab. Res. 9, 457–465.

Schatz, H., Laube, H., Sieradzki, J., Kamenisch, W., Pfeiffer, E.F., 1978. Long-term actions of sulphonylureas on (pro-) insulin biosynthesis and secretion: II. Studies after administration of tolbutamide and glibenclamide to rats in vivo. Horm. Metab. Res. 10, 23–29.

Sodoyez, J.-C., Sodoyez-Goffaux, F., Dunbar, J.C., Foa, P.P., 1970. Reduction in the activity of the pancreatic islets induced in normal rodents by prolonged treatment with derivatives of sulphonylurea. Diabetes 19, 603–609.

Tian, Y.A., Johnson, G., Ashcroft, S.J., 1998. Sulfonylureas enhance exocytosis from pancreatic beta-cells by a mechanism that does not involve direct activation of protein kinase C. Diabetes 47, 1722–1726.