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Induced desensitization of the insulinotropic effects of antidiabetic drugs, BTS 67 582 and tolbutamide

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1 Acute and chronic mechanisms of action of novel insulinotropic antidiabetic drug, BTS 67 582 (1,1-dimethyl-2-(2-morpholinophenyl)guanidine fumarate), were examined in the stable cultured BRIN-BD11 cell line.

2 BTS 67 582 (100–400 μM) stimulated a concentration-dependent increase ($P<0.01$) in insulin release at both non-stimulatory (1.1 mM) and stimulatory (8.4 mM) glucose.

3 Long-term exposure (3–18 h) to 100 μM BTS 67 582 in culture time-dependently decreased subsequent responsiveness to acute challenge with 200 μM BTS 67 582 or 200 μM tolbutamide at 12–18 h ($P<0.001$). Similarly 3–18 h culture with the sulphonylurea, tolbutamide (100 μM), also effectively suppressed subsequent insulinotropic responses to both BTS 67 582 and tolbutamide.

4 Culture with 100 μM BTS 67 582 or 100 μM tolbutamide did not affect basal insulin secretion, cellular insulin content, or cell viability and exerted no influence on the secretory responsiveness to 200 μM of the imidazoline, efaroxan.

5 While 18 h BTS 67 582 culture did not affect the insulin-releasing actions ($P<0.001$) of 16.7 mM glucose, 10 mM arginine, 30 mM KCl, 25 μM forskolin or 10 nM phorbol-12-myristate 13-acetate (PMA), significant inhibition ($P<0.001$) of the insulinotropic effects of 10 mM 2-ketoisocaproic acid (KIC) and 10 mM alanine were observed.

6 These data suggest that BTS 67 582 shares a common signalling pathway to sulphonylurea but not imidazoline drugs. Desensitization of drug action may provide an important approach to dissect sites of action of novel and established insulinotropic antidiabetic agents.
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Abbreviations: BTS 67 582, 1,1-dimethyl-2-(2-morpholinophenyl)guanidine fumarate; cyclic AMP, 3′: 5′-cyclic monophosphate; K_{ATP} channel, ATP-sensitive-K^+ channel; KIC, 2-ketoisocaproic acid; PLC, phospholipase C; PKA, protein kinase A; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; VDCC, voltage-dependent calcium channel

Introduction

The guanidine-derivative, BTS 67 582 (1,1-dimethyl-2-(2-morpholinophenyl)guanidine fumarate), represents one of a new class of antidiabetic agents (Bailey *et al*., 1997). In addition to an ability to lower blood glucose in normal and animal models of type 2 diabetes (Kaul *et al*., 1995; Jones *et al*., 1997; Page & Bailey, 1997; Storey & Bailey, 1998), BTS 67 582 has also been demonstrated to exert similar effects in both normal and diabetic humans (Byrom *et al*., 1994; 1997; Skillman & Raskin, 1997). As a result of these actions, due primarily to elevations of insulin concentrations, BTS 67 582 has been proposed as a clinically useful novel oral antidiabetic drug.

Sulphonylureas remain the most important class of insulinotropic drug currently used in type 2 diabetes therapy (DeFronzo, 1998). These drugs are known to exert direct actions on the pancreatic beta cell through binding to the sulphonylurea receptor subunit (SUR1) of the two component ATP-sensitive-K^+ (K_{ATP}) channel complex (Ashcroft & Gribble, 1998; Ashfield *et al*., 1999; Aguilar-Bryan & Bryan, 1999). This elicits membrane depolarization and elevation of cytoplasmic Ca^{2+} due to increased Ca^{2+} influx through voltage-dependent calcium channels (VDCC) (Nelson *et al*. 1992; Dunne *et al*., 1994; Ashcroft & Gribble, 1998). In addition to their ability to directly induce insulin release, sulphonylureas can also potentiate the insulinotropic actions of glucose, amino acids and 3′: 5′-cyclic monophosphate (cyclic AMP) (Ostenson *et al*., 1983; Sako *et al*., 1986). While plasma membrane SUR1 is the primary target of sulphonylurea action, additional intracellular sites are thought to exist through which these drugs may elicit insulin secretion in the absence of K_{ATP} channel function (McClenaghan & Flatt, 1999a).

A number of other potentially important oral antidiabetic agents also act through targeting the K_{ATP} channel including: imidazolines (e.g. efaroxan, phentolamine); the phenylalanine-dervivative, A-4166; and the benzoic acid-derivative, repaglinide (Chan *et al*., 1991; Akiyoski *et al*., 1995; Rustenbeck *et al*., 1995; Zaitsev *et al*., 1996; Proks & Ashcroft, 1997; Fuhlendorff *et al*., 1998). Closure of the K_{ATP} channel by these structurally diverse drugs is believed to result primarily from direct interaction with the channel pore (Kir6.2) and/or SUR1 subunits (Chan *et al*., 1991; Akiyoski *et al*., 1995; Rustenbeck *et al*., 1995; Zaitsev *et al*., 1996; Proks & Ashcroft, 1997; Fuhlendorff *et al*., 1998). Characterization of the principal mechanisms underlying the insulin-releasing actions of BTS 67 582 also identified the K_{ATP} channel as the primary site of action (Dickinson *et al*., 1997; Louchami *et al*., 1998; McClenaghan *et al*., 1998c). However, while previous studies indicate common signalling pathways utilized by BTS 67 582 and sulphonylureas (McClenaghan *et al*., 1998c), relatively

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little is known to date about the precise sites of action of BTS 67 582 in the pancreatic beta cell. Recent observations indicate that unlike sulphonylureas, which stimulate a characteristic monophasic insulinotropic response, BTS 67 582 may exert a biphasic insulin-releasing effect (Storey & Bailey, 1998).

Chronic hyperglycaemia in type 2 diabetes is associated with glucose desensitization and glucose toxicity (Ward *et al*., 1984; Portha *et al*., 1994; Ammon, 1997; Yki-Jarvinen, 1998; Zawalich *et al*., 1998). While glucose desensitization has obvious implications in type 2 diabetes, this phenomenon may also extend to other physiological and pharmacological secretagogues, including the sulphonylureas (Grunberger, 1993; Morgan *et al*., 1994; Chapman *et al*., 1999). Indeed, the decline in sulphonylurea activity during long term application has been suggested to be directly attributable to a densensitization of the pancreatic beta cell to the action of these drugs (Dunbar & Foa, 1974; Filiponni *et al*., 1983; Karam *et al*., 1986; Grunberger, 1993). Due to the widespread use of these drugs, it is perhaps surprising that little attention has been devoted to the mechanisms underlying sulphonylurea densensitization (Gullo *et al*., 1991; Rabuazzo *et al*., 1992).

The present study exploits the stable cultured pancreatic BRIN-BD11 cell line (McClenaghan *et al*., 1996a) to study the acute and chronic mechanisms of action of BTS 67 582 on the pancreatic beta cell. These results demonstrate for the first time that prolonged exposure to BTS 67 582 can induce desensitization to the acute actions of both BTS 67 582 and tolbutamide. Distinct and common modes of action between BTS 67 582, tolbutamide and efaroxan are revealed together with consequences of BTS 67 582 desensitization on the regulation of insulin secretion by other agents. In addition, this study highlights how induction of drug desensitization may be a useful model system to discriminate between the modes of action of a range of therapeutically relevant insulinotropic agents.

Methods

Clonal pancreatic BRIN-BD11 cells (passage 28–35) were used in this study. Characteristics of this glucose-responsive cell line, derived from electrofusion of RINm5F cells with rat pancreatic beta cells, have been detailed elsewhere (McClenaghan *et al*., 1996a,b,c, 1998b; McClenaghan & Flatt, 1999a). BRIN-BD11 cells typically retain their functional features in extended culture and thus offer an attractive alternative to cultured pancreatic beta cells, which exhibit an inherently short functional lifespan.

BRIN-BD11 cells were grown in RPMI-1640 tissue culture medium containing 11.1 mM glucose, 0.3 g l^{-1}1 L-glutamine, and supplemented with 10% (v v^{-1}) foetal calf serum, 100 IU ml^{-1} penicillin and 0.1 g l^{-1} streptomycin (Gibco, Paisley, Strathclyde, U.K.). Cells were washed with Hank's balanced saline solution prior to detachment from tissue culture flasks with the aid of 0.025% trypsin containing 1 mM EDTA. Cells were then seeded at 1.5×10^5 cells per well into 24-multiwell plates (Nunc, Rolkilde, Denmark). Monolayers of BRIN-BD11 cells were cultured (3–18 h) in the absence (standard culture conditions) or presence of either 100 μM BTS 67 582 (Knoll Pharmaceuticals Research and Development, Nottingham, U.K.) or tolbutamide (Sigma Chemical Company, Poole, U.K.) at 37°C. After the appropriate period, culture medium was replaced with 1 ml of a Krebs Ringer Bicarbonate (KRB) buffer supplemented with 0.1% bovine serum albumin and 1.1 mM glucose (McClenaghan *et al*., 1996c). Following a 40 min preincubation step at 37°C, to allow the cells to equilibrate with the KRB buffer, the preincubation buffer was replaced with 1 ml of KRB test buffer containing 1.1 or 8.4 mM glucose plus test agents as detailed in the legends to the Figures. After 20 min incubation at 37°C, aliquots of test buffer were removed and stored at −20°C for insulin radioimmunoassay (Flatt & Bailey, 1981). Insulin release (ng per 10^6 cells 20 min^{-1}) is expressed as mean ± standard error of the mean (s.e.mean) of six independent observations. Groups were compared by Student's unpaired *t*-test and differences considered significant if $P<0.05$.

Results

Insulinotropic responses at non-stimulatory and stimulatory glucose concentrations

Acute static incubation with 100–400 μM BTS 67 582 (EC_{50} 150) evoked a 1.2–2.6 fold concentration-dependent increase ($P<0.01$ to $P<0.001$) in insulin release at non-stimulatory (1.1 mM) and stimulatory (8.4 mM) glucose (Figure 1). Characteristic of the BRIN-BD11 cells, raising the glucose concentration from 1.1 to 8.4 mM stimulated a 1.6 fold ($P<0.001$) insulin-secretory response, and resulted in an additive 1.1–1.5 fold ($P<0.05$ to $P<0.001$) potentiation of BTS 67 582-induced insulin release (Figure 1). BTS 67 582 exhibited a similar secretory profile to tolbutamide (maximal response 400 μM; EC_{50} 160; unpublished observations) consistent with an almost equipotent effect in rat islets (Louchami *et al*., 1998).

Responsiveness to insulinotropic drugs following exposure to BTS 67 582 and tolbutamide in culture

As shown in Figure 2a, BTS 67 582, efaroxan and tolbutamide (each at 200 μM) elicited respective 2.1-, 2.5- and 1.9 fold ($P<0.001$) insulin secretory responses from the BRIN-BD11 cells after culturing for 18 h in standard RPMI-1640 media. However, 18 h culture with 100 μM BTS 67 582 abolished subsequent secretory responses to both BTS 67 582 and tolbutamide (Figure 2a). Notably the response to efaroxan was unaffected (Figure 2a), indicating different sites of action of BTS 67 582 and imidazoline drugs. A similar pattern

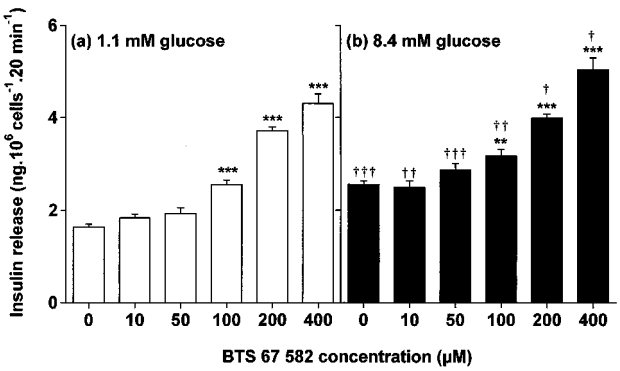

Figure 1 Effects of 0–400 μM BTS 67 582 at (a) non-stimulatory (1.1 mM) or (b) stimulatory (8.4 mM) glucose. Following 40 min of preincubation with a buffer containing 1.1 mM glucose, effects of BTS 67 582 were tested during a 20 min incubation period. Values are mean ± s.e.mean for six separate observations. $^{**}P<0.01$, $^{***}P<0.001$ compared with respective effects in the absence of BTS 67 582. $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$ compared with respective effects at 1.1 mM glucose.

emerged following 18 h culture with 100 μM tolbutamide, with an abolition of both BTS 67 582 and tolbutamide-induced insulin release, whilst the insulinotropic actions of efaroxan remained intact (Figure 2b).

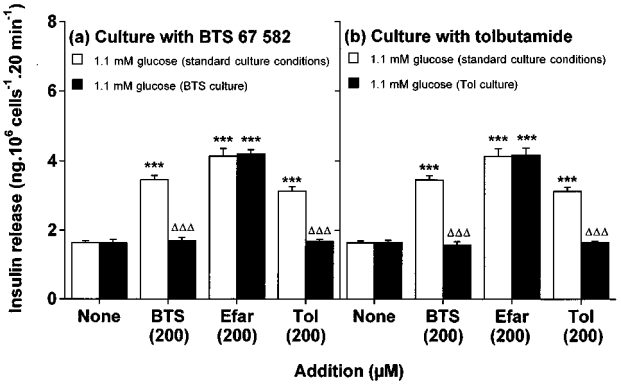

Figure 2 Effects of culture with (a) BTS 67 582 or (b) tolbutamide on BTS 67 582-, efaroxan- and tolbutamide-induced insulin secretion. After 18 h culture in the absence (standard culture conditions) or presence of either 100 μM BTS 67 582 (BTS culture) or 100 μM tolbutamide (Tol culture), cells were preincubated for 40 min before 20 min acute incubation with a buffer containing 1.1 mM glucose in the absence or presence of 200 μM BTS 67 582 (BTS), 200 μM efaroxan (Efar) or 200 μM tolbutamide (Tol). Values are mean$\pm$s.e.mean for six separate observations. ***$P<0.001$ compared with respective effects in the absence of addition. $\Delta\Delta\Delta P<0.001$ compared with respective effects after standard culture conditions.

Time-dependency of downregulation of insulinotropic actions of BTS 67 582 and tolbutamide

Subsequent responsiveness to BTS 67 582 or tolbutamide was examined following 3, 6, 12 and 18 h to examine the possible time-dependency of downregulation. As shown in Figure 3a, there was a progressive decline in the insulin secretory effects of BTS 67 582 following 3–18 h culture with 100 μM BTS 67 582, reaching significance at 12 h (61% decrease, $P<0.001$) and with a maximum suppression (84% decrease, $P<0.001$) by 18 h. Subsequent responsiveness to tolbutamide after 3–18 h exposure to BTS 67 582 (Figure 3b) followed a similar pattern, also reaching significance at 12 h (73% decrease, $P<0.001$) and with a maximal reduction (95% decrease, $P<0.001$) by 18 h. As shown in Figure 3c, 12–18 h culture with 100 μM tolbutamide was also effective at suppressing (by 89–96%, $P<0.001$) subsequent responsiveness to BTS 67 582. These conditions similarly suppressed (by 44–58%, $P<0.01$ to $P<0.001$) the subsequent insulinotropic action of tolbutamide (data not shown). Culture for 3–18 h with BTS 67 582 or tolbutamide did not affect basal insulin secretion (at 1.1 mM glucose), cellular insulin content (64–71 ng 10^6 cells^{-1}), or cell viability (assessed using trypan blue). Prolonged exposure to efaroxan (6–18 h) did not alter subsequent secretory responsiveness to either BTS 67 582 or tolbutamide (unpublished observations).

Reversibility of downregulation of insulinotropic actions of BTS 67 582

Possible recovery of the insulinotropic capacities of BTS 67 582 and tolbutamide after drug exposure were assessed after

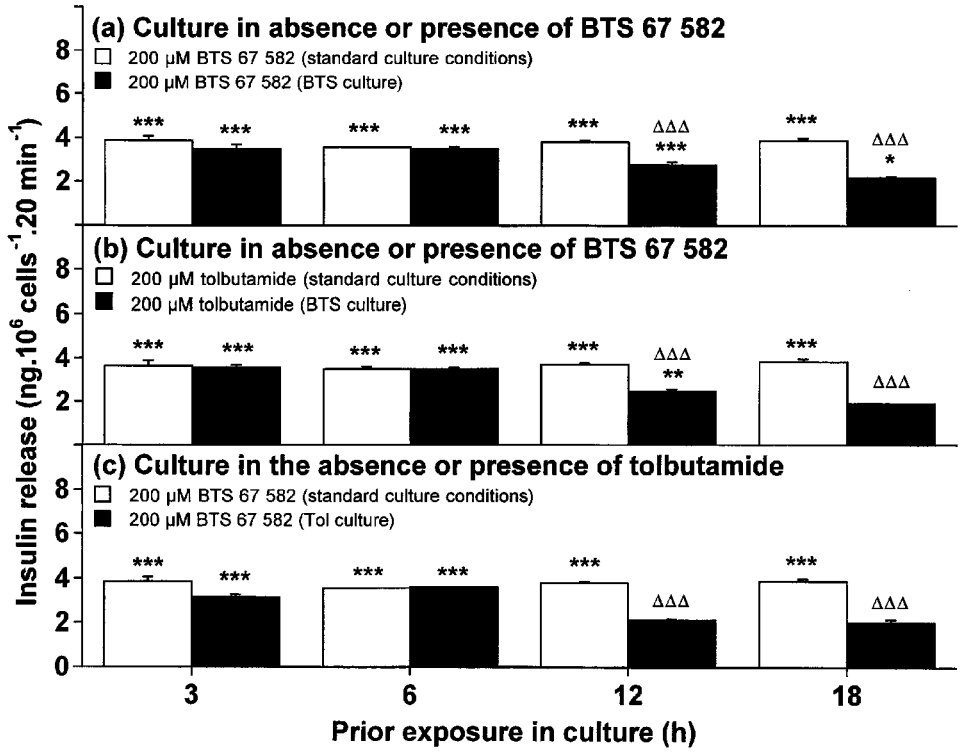

Figure 3 Time-dependent effects of culture with (a,b) BTS 67 582 or (c) tolbutamide on subsequent insulin-secretory responsiveness to (a,c) BTS 67 582 and (b) tolbutamide. After 3, 6, 12 or 18 h culture in the absence (standard culture conditions) or presence of either 100 μM BTS 67 582 (BTS culture) or 100 μM tolbutamide (Tol culture), cells were preincubated for 40 min before 20 min acute incubation with a buffer containing 1.1 mM glucose in the absence or presence of either 200 μM BTS 67 582 (BTS) or 200 μM tolbutamide (Tol). Values are mean$\pm$s.e.mean for six separate observations. Insulin-secretory responses to control (1.1 mM glucose) remained constant from 3–18 h regardless of culture condition employed (in ng per 10^6 cells 20 min^{-1}: 1.90 ± 0.11 for standard culture conditions; 1.97 ± 0.12 for BTS culture; 1.88 ± 0.09 for Tol culture). *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with respective effects of control (1.1 mM glucose alone). $\Delta\Delta\Delta P<0.001$ compared with respective effects after standard culture conditions.

recovery periods of 6 and 12 h. Returning BRIN-BD11 cells to standard culture conditions following 18 h culture with 100 μM BTS 67 582 resulted in a 418% increase ($P<0.001$; 56% of maximal response) insulin-secretory responsiveness to BTS 67 582 at 6 h, with a complete recovery (712% increase, $P<0.001$) at 12 h (Figure 4a). Likewise, a significant 326% increase ($P<0.001$; 43% of maximal response) in secretory responsiveness to tolbutamide was observed at 6 h, with full responsiveness (667% increase, $P<0.001$) being achieved 12 h post-BTS 67 582 culture (Figure 4b). After tolbutamide culture, secretory responsiveness to BTS 67 582 returned at 6 h (544% increase, $P<0.001$; 55% of maximal response) with a complete restoration (908% increase, $P<0.001$) at 12 h (Figure 4c). Secretory responsiveness to tolbutamide similarly

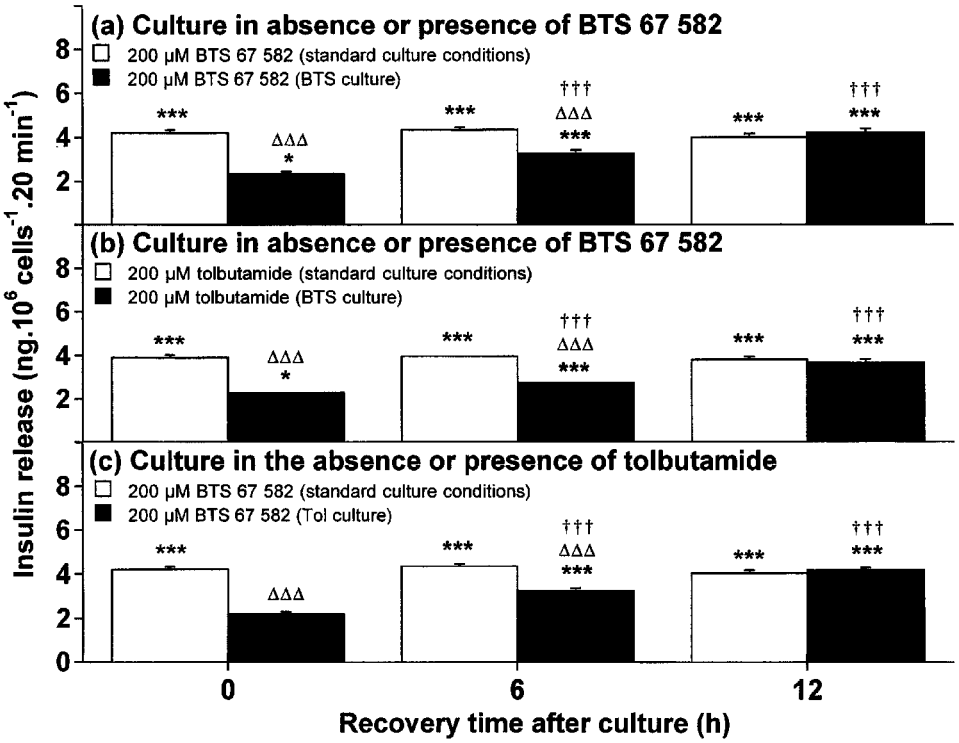

Figure 4 Time-dependent recovery of insulin-secretory responsiveness to (a,c) BTS 67 582 or (b) tolbutamide after 18 h culture with (a,b) 100 μM BTS 67 582 or (c) tolbutamide. After 18 h culture in the absence (standard culture conditions) or presence of either 100 μM BTS 67 582 (BTS culture) or 100 μM tolbutamide (Tol culture), cells were cultured in the absence of BTS 67 582 or tolbutamide for 0, 12 or 18 h before preincubation (40 min) and subsequent 20 min incubation with a buffer containing 1.1 mM glucose in the absence or presence of 200 μM BTS 67 582 (BTS) or 200 μM tolbutamide (Tol). Values are mean$\pm$s.e.mean for six separate observations. Insulin-secretory responses to control (1.1 mM glucose) remained constant from 3–18 h regardless of culture condition employed (in ng per 10^6 cells 20 min^{-1}: 1.92$\pm$0.08 for standard culture conditions; 2.00$\pm$0.08 for BTS culture; 1.96$\pm$0.11 for Tol culture). *$P<0.05$, ***$P<0.001$ compared with respective effects of control (1.1 mM glucose). $\Delta\Delta\Delta P<0.001$ compared with respective effects after standard culture conditions. †††$P<0.001$ compared with respective effects at 0 h.

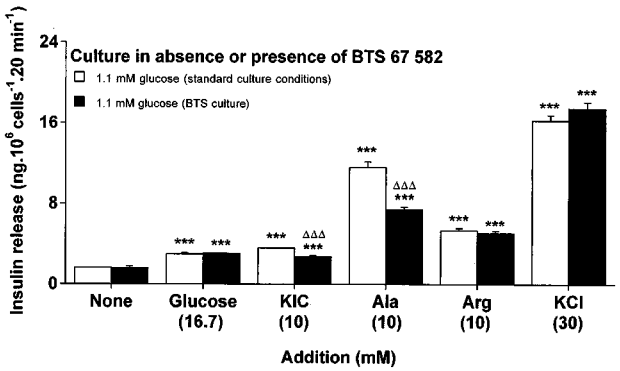

Figure 5 Effects of culture with BTS 67 582 on insulin secretory responses to nutrient secretagogues and KCl. After 18 h culture in the absence or presence of 100 μM BTS 67 582 (BTS culture), cells were preincubated for 40 min before 20 min acute incubation with a buffer containing 1.1 mM glucose in absence or presence of 16.7 mM glucose, 10 mM 2-ketoisocaproic acid (KIC), 10 mM L-alanine (Ala), 10 mM L-arginine (Arg) or 30 mM KCl. Values are mean$\pm$s.e.mean for six separate observations. ***$P<0.001$ compared with respective effects in the absence of addition. $\Delta\Delta\Delta P<0.001$ compared with respective effects after standard culture conditions.

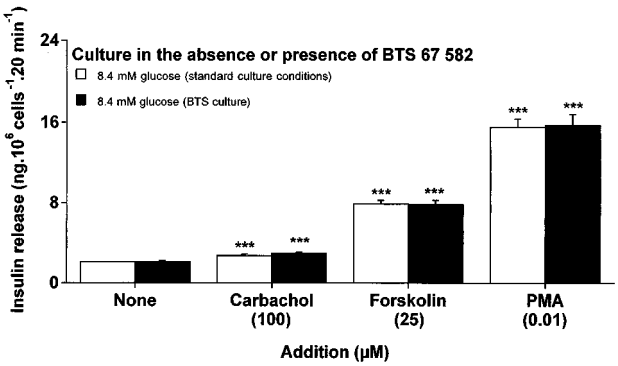

Figure 6 Effects of culture with BTS 67 582 on insulin secretory responses to carbachol, forskolin or phorbol-12-myristate 13-acetate (PMA). After 18 h culture in the absence or presence of 100 μM BTS 67 582 (BTS culture), cells were preincubated for 40 min before 20 min acute incubation with a buffer containing 8.4 mM glucose in absence or presence of 100 μM carbachol, 25 μM forskolin or 10 nM PMA. Values are mean$\pm$s.e.mean for six separate observations. ***$P<0.001$ compared with respective effects in the absence of addition.

returned at 6 h ($P<0.001$) and was fully restored at 12 h (unpublished observations).

Downregulation with BTS 67 582 on actions of nutrient insulin secretagogues and KCl

Insulinotropic responses to stimulatory (16.7 mM) glucose, 10 mM 2-ketoisocaproic acid (KIC), 10 mM alanine, 10 mM arginine or 30 mM KCl were examined after 18 h culture in the absence or presence of 100 μM BTS 67 582. As shown in Figure 5, raising the glucose concentration from 1.1 to 16.7 mM glucose stimulated a 1.8 fold increase in insulin secretion, which remained unaffected after BTS 67 582 culture. The 2-keto acid, KIC stimulated a 2.2 fold ($P<0.001$) insulin secretory response which was inhibited (by 43%, $P<0.001$) by BTS 67 582 culture (Figure 5). Likewise, alanine stimulated a 7.1 fold ($P<0.001$) insulin secretory response which was impaired (41% decrease, $P<0.001$) following culture with BTS 67 582 (Figure 5). Interestingly, the respective 3.3 and 9.9 fold responses to 10 mM arginine and 30 mM KCl remained unaffected (Figure 5), indicating that BRIN-BD11 cells were still able to exhibit appropriate responses to membrane depolarization after BTS 67 582 culture. Other studies have confirmed 18 h culture with 100 μM tolbutamide did not affect subsequent secretory responses to equimolar glucose, KIC, alanine or KCl (unpublished observations), indicating the complexity of the mechanisms underlying desensitization by drugs.

Effects of downregulation with BTS 67 582 culture on secretory pathways activated by carbachol, forskolin or PMA

Insulin secretory responsiveness to carbachol (100 μM), forskolin (25 μM) or phorbol 12-myristate 13-acetate (PMA; 10 nM) were assessed after 18 h culture in the absence or presence of 100 μM BTS 67 582. As shown in Figure 6, the 1.3, 3.7 and 7.4 fold insulin secretory responses to carbachol, forskolin and PMA were totally unaffected following BTS 67 582 culture, indicating that BRIN-BD11 cells retained intact phospholipase C (PLC), adenylate cyclase, protein kinase A (PKA) and protein kinase C (PKC) mediated signalling pathways.

Discussion

The present study examines the long-term effects and modes of action of the novel antidiabetic drug BTS 67 582, using cultured clonal glucose-responsive insulin-secreting cells. The BRIN-BD11 cell line has been thoroughly characterized and displays appropriate secretory responses to a wide range of physiological and pharmacological regulators of insulin release (see McClenaghan *et al*., 1996a,b,c; McClenaghan & Flatt, 1999a,b). These attributes combined with a high degree of functional stability, make such cells particularly useful for evaluation of longer-term effects of drugs on pancreatic beta cell function in culture.

Consistent with previous observations, BTS 67 582 acted as both an initiator and potentiator of insulin release from BRIN-BD11 cells (McClenaghan *et al*., 1998c). In the presence of both non-stimulatory (1.1 mM) or stimulatory (8.4 mM) glucose concentrations, BTS 67 582 elicited insulin-secretory responses in a concentration-dependent manner over the range 100–400 μM. These data support studies using perifused rat islets which demonstrated notable insulin-releasing effects in the presence of physiological concentrations of glucose (5–8 mM) (Dickinson *et al*., 1997). BRIN-BD11 cells also responded to two other important classes of antidiabetic agent, the imidazoline drug, efaroxan, and the sulphonylurea, tolbutamide; and it is notable that equimolar amounts of each of these structurally diverse compounds had similar insulin-releasing potencies.

Prolonged use of sulphonyureas in type 2 diabetes is often associated with a progressive decline in their glucose-lowering ability (Grunberger, 1993; Harrower, 1994; Pontiroli *et al*., 1994). This phenomenon has been attributed to a progressive desensitization of the pancreatic beta cells to sulphonylurea action (Dunbar & Foa, 1974; Filiponni *et al*., 1983; Karam *et al*., 1986; Grunberger, 1993). The present study evaluated whether this phenomenon could be induced *in vitro* and exploited to study the site(s) of action of BTS 67 582 and other important antidiabetic drugs. New evidence is provided indicating desensitization to BTS 67 582 by 18 h exposure to this drug. Furthermore, culture with BTS 67 582 culture also induced desensitization to insulinotropic effects tolbutamide, but not efaroxan. Sulphonylurea desensitization could also be induced by 18 h culture of BRIN-BD11 cells with tolbutamide, indicating no particular advantage or disadvantage with either class of drug. The insulinotropic action of BTS 67 582 was also desensitized by sulphonylurea exposure, suggestive of common sites of action. The inability of either drug in culture to affect efaroxan-induced insulin secretion clearly indicates distinct and novel actions of imidazolines (Morgan *et al*., 1994; Efanov *et al*., 1998; Chapman *et al*., 1999).

Taken together, these observations support the notion (McClenaghan *et al*., 1998c) that BTS 67 582 exerts its primary effects through similar signalling pathways to tolbutamide, extending this view to suggest diverse sites of interaction from efaroxan. Notably, the induction of desensitization by BTS 67 582 followed a time-dependent pattern becoming significant after 12 h exposure in culture, with a maximum inhibition of both BTS 67 582- and tolbutamide-induced insulin release by 18 h. Similarly, exposure of BRIN-BD11 cells to 12 h tolbutamide culture significantly reduced the insulinotropic response to BTS 67 582, with an abolition of BTS 67 582-induced insulin secretion after 18 h tolbutamide culture. Reversibility of drug desensitization was inducible by exposing desensitized cells to culture in the absence of BTS 67 582 or tolbutamide. In each case, BTS 67 582 or tolbutamide desensitization of insulin secretion was significantly reversed after 6 h, with a full restoration of drug-induced secretory responsiveness by 12 h. These observations together with lack of effect of 18 h culture on basal insulin secretion or cellular insulin content argue against a simple toxic action of these drugs.

Additional experiments were perfomed to examine the nature of BTS 67 582 desensitization, and its impact on the actions of established secretagogues. Consistent with previous observations (McClenaghan *et al*., 1996a,b; 1998a; McClenaghan & Flatt, 1999a,b), BRIN-BD11 cells exposed to standard culture conditions showed notable insulin secretory responses to glucose, KIC, alanine, arginine and 30 mM KCl. Interestingly, BTS 67 582 desensitization was associated with a reduction in the secretory activity of the metabolizable nutrients KIC and alanine but not that of glucose or the depolarizing actions of either arginine or KCl. However, BTS 67 582 desensitization did not alter the actions of carbachol, forskolin or PMA indicating intact PLC-, PKA- and PKC-mediated signal function, respectively. Taken together these data indicate that BTS 67 582 desensitization,

while not generally affecting late steps in stimulus secretion coupling, may share and hence alter common signalling pathways to KIC and alanine.

These actions may reflect a change in beta cell function relating to the regulation of K_{ATP} channel activity perhaps, in the case of metabolizable amino acids, mediated at a mitochondrial level, resulting in the alteration of metabolite generation and/or action on the channel. When considering the present data it is important to note that in addition to affecting the beta cell stimulus secretion pathway through ATP generation, KIC can also directly inhibit the K_{ATP} channel (Branstrom *et al*., 1998). While being cautious not to overinterpret these findings, is interesting to speculate that BTS 67 582 desensitization, in addition to influencing its own binding and hence secretory function, could also possibly interfere with direct actions of KIC and other compounds on the K_{ATP} channel.

In conclusion, while the exact site(s) of action of BTS 67 582 remains to be established, the present data support the view that BTS 67 582 and tolbutamide share common beta cell signalling pathways. This study also demonstrates for the first time the induction of BTS 67 582 densensitization in a clonal beta cell line and, as indicated by Chapman *et al*. (1999), elucidation of the mechanisms underlying desensitization by drugs is not straightforward. In addition to providing a unique model system in which to study the mechanism and sites of action of BTS 67 582, future studies utilizing this novel approach should help unravel the complex signalling pathways utilized by a spectrum of clinically relevant insulinotropic drugs.

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