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Drug-Induced Desensitization of Insulinotropic Actions of Sulfonylureas

Andrew J. Ball, Jane T. McCluskey, Peter R. Flatt, and Neville H. McClenaghan[1]
School of Biomedical Sciences, University of Ulster, Coleraine, BT52 1SA, Northern Ireland, United Kingdom

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K_{ATP}-channel-dependent and K_{ATP}-channel-independent insulin-releasing actions of the sulfonylurea, tolbutamide, were examined in the clonal BRIN-BD11 cell line. Tolbutamide stimulated insulin release at both nonstimulatory (1.1 mM) and stimulatory (16.7 mM) glucose. Under depolarizing conditions (16.7 mM glucose plus 30 mM KCl) tolbutamide evoked a stepwise K_{ATP} channel-independent insulinotropic response. Culture (18 h) with tolbutamide or the guanidine derivative BTS 67 582 (100 μM) markedly reduced ($P < 0.001$) subsequent responsiveness to acute challenge with tolbutamide, glibenclamide, and BTS 67 582 but not the imidazoline drug, efaroxan. Conversely, 18 h culture with efaroxan reduced ($P < 0.001$) subsequent insulinotropic effects of efaroxan but not that of tolbutamide, glibenclamide, or BTS 67 582. Culture (18 h) with tolbutamide reduced the K_{ATP} channel-independent actions of both tolbutamide and glibenclamide. Whereas culture with efaroxan exerted no effect on the K_{ATP} channel-independent actions of sulfonylureas, BTS 67 582 abolished the response of tolbutamide and inhibited that of glibenclamide. These data demonstrate that prolonged exposure to tolbutamide desensitizes both K_{ATP}-channel-dependent and -independent insulin-secretory actions of sulfonylureas, indicating synergistic pathways mediated by common sulfonylurea binding site(s).

Sulfonylureas remain the most important class of insulinotropic antidiabetic drug used to treat type 2 diabetes (1, 2). Molecular and functional studies have established that these drugs exert direct actions on the pancreatic β cell principally by binding to the sulfonylurea receptor subunit (SUR1) of the two-component ATP-sensitive K^+ (K_{ATP}) channel (3–6). Sulfonylurea binding to the K_{ATP} channel elicits membrane depolarization which opens of voltage-dependent Ca^{2+} channels (VDCC) resulting in an elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and ultimately insulin exocytosis (6–8).

Although the *in vivo* and *in vitro* insulinotropic actions of sulfonylureas are undisputed, the precise mechanisms underlying the more diverse effects of this important class of drugs on pancreatic β cells remains a key focus of current research (8–13). While SUR1 is the primary target of sulfonylurea action, the complex nature of β cell sulfonylurea-signaling is highlighted by potentiating effects on glucose-, amino acid- and cyclic adenosine 3′:5′-monophosphate (cAMP)-induced insulin release (14, 15) and the recently proposed K_{ATP} channel-independent effects of sulfonylureas (8–13).

Chronic hyperglycemia characterizing type 2 diabetes is associated with glucose desensitization and glucose toxicity (16, 17). An increasing body of evidence suggests that this desensitization phenomenon may extend to include a number of other important physiological and pharmacological modulators of β cell function. Indeed, despite obvious implications in both the progression and treatment of type 2 diabetes, mechanisms underlying the decline in sulfonylurea activity associated with long-term application has received surprisingly little attention to date (1, 18–21).

The study and interpretation of long-term effects of physiological and pharmacological agents on pancreatic β cells has been complicated by phenotypic instability and the relatively short functional life-span of pancreatic β cells *in vitro*. The recent emergence of stable pancreatic β cell lines with intact responses to glucose and other secretagogues (8, 22, 23) greatly facilitates such research. The present study exploits one such clonal β cell model to examine the possible desensitization of K_{ATP} channel-dependent and K_{ATP} channel-independent actions of sulfonylureas following long-term exposure to the popular first-generation sulfonylurea, tolbutamide. Additional studies focus on the effects of prolonged exposure to two other clinically relevant oral insulinotropic agents (efaroxan and BTS 67 582) also known to target the K_{ATP} channel and

[1] To whom correspondence should be addressed. Fax: +44-(0)2870-324965. E-mail: nh.mcclenaghan@ulst.ac.uk.

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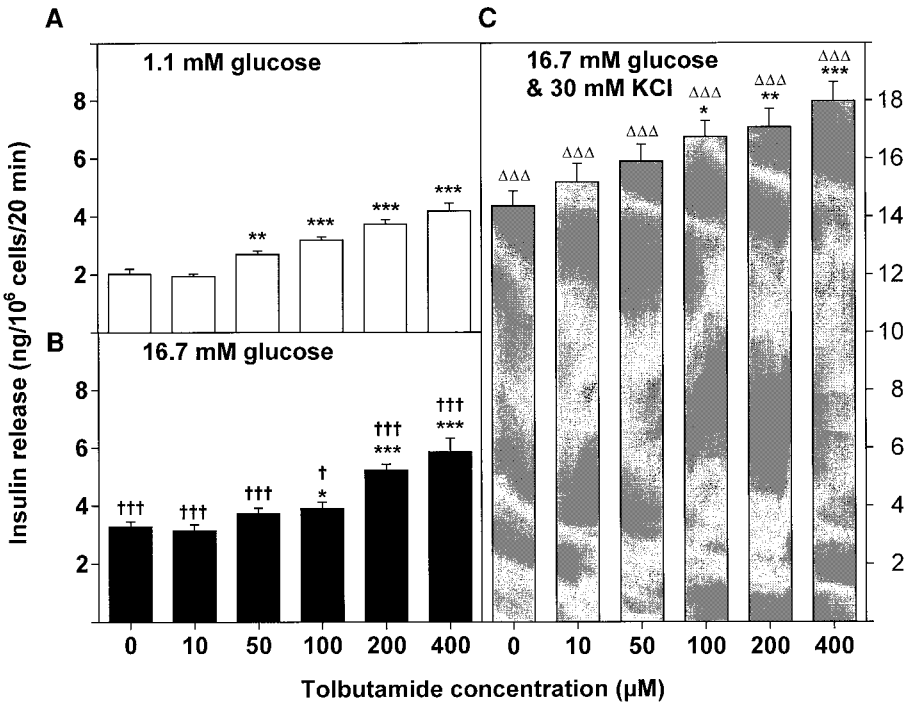

FIG. 1. Effects of 0–400 μM tolbutamide at (A) nonstimulatory (1.1 mM) or (B) stimulatory (16.7 mM) glucose or (C) in the presence of membrane depolarizing conditions (16.7 mM glucose plus 30 mM KCl). Following 40 min of preincubation with a buffer containing 1.1 mM glucose, effects of tolbutamide were tested during a 20-min incubation period. Values are means ± SEM for 6 separate observations. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with respective effects in the absence of tolbutamide. Insulin secretion in the presence of 16.7 mM glucose plus 30 mM KCl were constantly greater ($P < 0.001$) than at 1.1 mM glucose. $^{\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.001$ compared with respective effects at 1.1 mM glucose. $^{\Delta\Delta\Delta}P < 0.001$ compared with respective effects at 16.7 mM glucose alone.

evoke insulin secretion both *in vitro* and *in vivo* (24–27).

MATERIALS AND METHODS

Chemicals. Reagents of analytical grade and deionized water (Purite, Oxon, UK) were used. Sulfonylureas and all other reagents were obtained from Sigma Chemical Co. Ltd (Poole, Dorset, UK), unless otherwise indicated.

Cell culture. BRIN-BD11 cells (28) were maintained at 11.1 mM glucose in tissue culture in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum, 100 IU/ml penicillin and 0.1 mg/ml streptomycin (Gibco) in a 37°C incubator (5% CO_2 and 95% air).

Insulin secretion. Cells were harvested using trypsin, seeded at a density of 1.5×10^5/well in 24 well dishes (Nunc, Roskilde, Denmark), and allowed to attach overnight at 37°C. After culture (3–18 h) in the absence (normal culture conditions) or presence of 100 μM tolbutamide, 100 μM efaroxan or 100 μM BTS 67 582 (1,1-dimethyl-2-(2-morpholinophenyl)guanidine fumarate, Knoll Pharmaceuticals Research and Development, Nottingham, UK), culture medium was removed and cell monolayers were subjected to a 40 min preincubation (37°C) with 1 ml Krebs Ringer Bicarbonate buffer (KRB; consisting of 115 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.28 mM $CaCl_2$, 1.2 mM KH_2PO_4, 25 mM Hepes and 8.4% (w/v) $NaHCO_3$, pH 7.4) supplemented with 0.1% bovine serum albumin and 1.1 mM glucose. Insulin secretion was assessed during a subsequent 20 min incubation with KRB test buffer supplemented with glucose and other agents as indicated in the figures. Test buffer was then removed from each well and aliquots stored at −20°C for insulin radioimmunoassay (28).

Statistical analyses. Data are expressed as means ± SEM. Statistical analyses were performed using Student's *t*-test. Groups were considered to be significantly different if $P < 0.05$.

RESULTS AND DISCUSSION

Previous studies have established that the insulinotropic actions of sulfonylurea are primarily mediated by binding plasma membrane β cell K_{ATP} channels (3–6). However, an increasing body of evidence suggests that, like glucose, certain physiological and pharmacological agents may exert additional insulinotropic actions not dependent on the modulation of K_{ATP} channel activity (8–13, 29–33). The present study utilizes the islet-derived glucose-responsive clonal BRIN-BD11 cell line (28, 34–36), previously shown to express the functional β cell K_{ATP} channel complex (37), in order to offer further insights into the insulinotropic actions of tolbutamide and other oral antidiabetic drugs, and to examine the phenomenon of drug-induced desensitization (18–21).

As shown in Fig. 1, raising the glucose concentration from 1.1 to 16.7 mM glucose evoked a significant 1.6-fold increase in insulin release ($P < 0.001$), characteristic of the BRIN-BD11 cells (8, 23). When tested at nonstimulatory (1.1 mM) glucose, 50–400 μM tolbut-

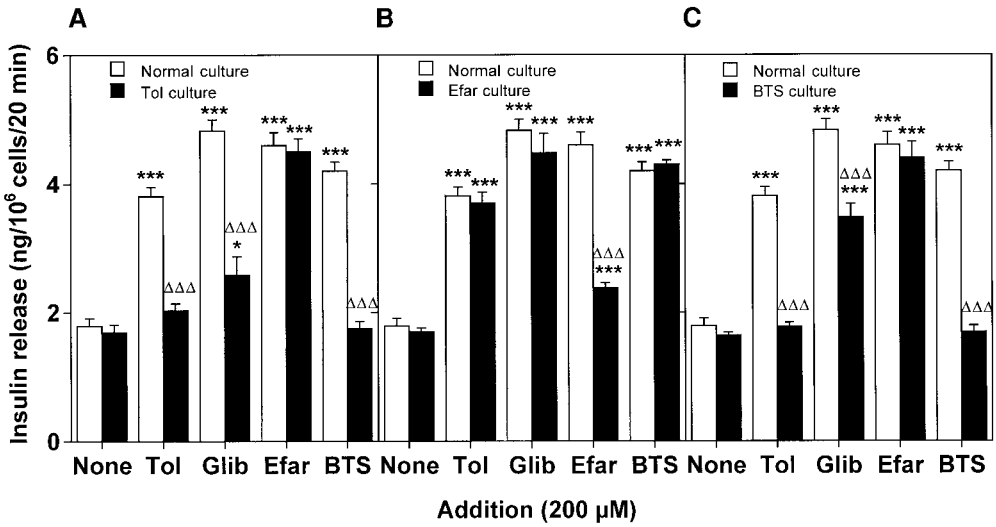

FIG. 2. Effects of culture with (A) tolbutamide, (B) efaroxan or (C) BTS 67 582 on tolbutamide-, glibenclamide-, efaroxan- and BTS 67 582-induced insulin secretion. After 18 h culture in the absence (normal culture conditions) or presence of 100 μM tolbutamide (Tol culture), 100 μM efaroxan (Efar culture) or 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 the absence or presence of 200 μM tolbutamide (Tol), 200 μM glibenclamide (Glib), 200 μM efaroxan (Efar), or 200 μM BTS 67 582 (BTS). Values are mean ± SEM for 6 separate observations. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with respective effects in the absence of addition. $^{\Delta\Delta\Delta}P < 0.001$ compared with respective effects after normal culture conditions.

amide initiated a stepwise increase (1.6- to 2.1-fold, $P < 0.01$ to $P < 0.001$) in insulin release (Fig. 1A). Similarly, at stimulatory (16.7 mM) glucose, 100–400 μM tolbutamide evoked a concentration-dependent 1.2- to 1.8-fold increase ($P < 0.05$ to $P < 0.001$) in insulin output (Fig. 1B).

Membrane depolarizing conditions were employed to further study the importance K_{ATP} channel-independent events in the regulation of glucose- and tolbutamide-induced insulin secretion. Under depolarizing conditions (16.7 mM glucose plus 30 mM KCl) sufficient to render K_{ATP} channels inactive (9–13, 30), tolbutamide (100–400 μM) stimulated 1.2- to 1.3-fold insulin-secretory responses (Fig. 1C). This extends K_{ATP} channel-independent insulinotropic actions of BRIN-BD11 cells, previously noted for glucose and 2-keto acids (31, 32), to include the sulfonylurea drugs.

Functional consequences of prolonged exposure to tolbutamide were examined with particular emphasis on establishing long-term effects on insulin secretory responses to clinically relevant pharmacological regulators of K_{ATP} channel activity. In accordance with the hypothesized desensitization of pancreatic β cells after prolonged exposure to sulfonylureas (18–21), 18 h culture with 100 μM of the first-generation sulfonylurea, tolbutamide, markedly decreased (by 47%, $P < 0.001$) subsequent acute insulin-releasing effects of this agent (Fig. 2A). Similarly, 18 h tolbutamide culture significantly decreased (by 47%, $P < 0.001$) insulin output in response to the second-generation sulfonylurea, glibenclamide (Fig. 2A). This further confirms that tolbutamide desensitization affects a common insulinotropic pathway utilized by other sulfonylurea drugs (9, 10, 18). Interestingly, prolonged exposure to tolbutamide, while exerting no influence on the insulinotropic imidazoline compound, efaroxan, effectively abolished BTS 67 582-induced insulin secretion. This is consistent with a common site of action of BTS 67 582 and sulfonylurea drugs (26).

These observations prompted additional studies to see if tolbutamide desensitization could be induced by other clinically relevant classes of insulinotropic drug acting through the K_{ATP} channel. Culture (18 h) with 100 μM efaroxan did not affect subsequent insulin-secretory actions of either sulfonylurea drug or indeed that of the guanidine-derivative, BTS 67 582 (Fig. 2B). However, 18 h efaroxan culture resulted in a significant (43%, $P < 0.001$) reduction in efaroxan-induced insulin secretion (Fig. 2B), indicating the specific nature of efaroxan-induced desensitization (38). In contrast, 18 h exposure to BTS 67 582, effectively removed the insulinotropic effects of equimolar concentrations of BTS 67 582 and tolbutamide, whilst leaving the secretory response to efaroxan completely intact (Fig. 2C). The partial inhibition of glibenclamide-induced insulin release under these circumstances might relate to its greater potency or ability to penetrate the β cell (39, 40). Collectively, these data demonstrate that the phenomenon of induced drug-desensitization extends to other classes of oral insulinotropic antidiabetic agents acting through direct modulation of K_{ATP} channel activity. Furthermore, the ability of sulfonylureas and BTS 67 582 to fully retain their secretory activity after efaroxan culture, and vice versa, demonstrates

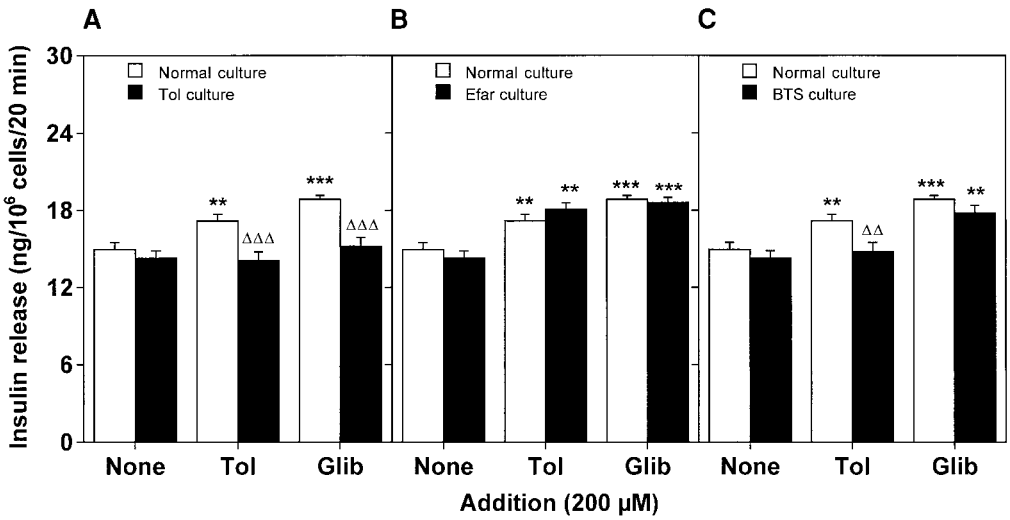

FIG. 3. Effects of culture with (A) tolbutamide, (B) efaroxan or (C) BTS 67 582 on insulinotropic actions of tolbutamide or glibenclamide under membrane depolarizing conditions. After 18 h culture in the absence (normal culture conditions) or presence of 100 μM tolbutamide (Tol culture), 100 μM efaroxan (Efar culture) or 100 μM BTS 67 582 (BTS culture), cells were preincubated for 40 min before 20 min acute incubation with a buffer containing 16.7 mM glucose and 30 mM KCl in the absence or presence of 200 μM tolbutamide (Tol) or 200 μM glibenclamide (Glib). Values are mean ± SEM for 6 separate observations. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with respective effects in the absence of addition. $^{\Delta\Delta}P < 0.01$, $^{\Delta\Delta\Delta}P < 0.001$ compared with respective effects after normal culture conditions.

that drug-induced desensitization cannot simply be attributed to K_{ATP} channel downregulation.

Further studies were designed to examine if sulfonylurea desensitization extended to insulinotropic actions not mediated through K_{ATP} channel-induced membrane depolarization. Following 18 h culture with 100 μM tolbutamide, acute effects of tolbutamide or glibenclamide (both at 200 μM) were tested in the presence of membrane depolarizing conditions (16.7 mM glucose plus 30 mM KCl). Similar experiments revealed that BTS 67 582 and efaroxan did not exhibit such effects (data not shown). As shown in Fig. 3A, tolbutamide culture removed subsequent K_{ATP} channel-independent insulin-secretory responses of tolbutamide and glibenclamide without significantly affecting membrane depolarization evoked by 16.7 mM glucose plus 30 mM KCl. As such, the present data indicate that prolonged exposure to sulfonylureas desensitizes both K_{ATP} channel-dependent and K_{ATP} channel-independent insulinotropic pathways without significantly altering membrane depolarization and hence K_{ATP} channel activity.

Given the selective nature of drug-densensitization it was of interest to determine if the K_{ATP} channel-independent responses to sulfonylureas persisted after 18 h culture with efaroxan or BTS 67 582 (both at 100 μM). As shown in Fig. 3B, efaroxan culture exerted no influence on the effects of tolbutamide or glibenclamide under membrane depolarizing conditions, suggesting diverse mechanisms regulating β cell imidazoline and sulfonylurea signaling. However, consistent with the view that BTS 67 582 shares common β cell actions with tolbutamide, prolonged exposure to this agent effectively removed the K_{ATP} channel-independent effects of tolbutamide (Fig. 3C). Interestingly, BTS 67 582 culture exerted a very modest effect on K_{ATP} channel-independent effects of glibenclamide (Fig. 3C), consistent with the inability of BTS 67 582 to completely inhibit the secretory effect of glibenclamide under nondepolarizing conditions (Fig. 2C).

Collectively the present data demonstrate that tolbutamide exerts both K_{ATP} channel-dependent and K_{ATP} channel-independent insulinotropic effects and prolonged exposure to tolbutamide in culture induces desensitization to both types of sulfonylurea action. The mechanisms behind K_{ATP} channel-independent effects of sulfonylureas and certain imidazolines remain to be established. However, recent studies have implicated late steps in the insulin secretory pathway, including effects mediated by protein kinase C and binding insulin secretory granules (8, 12, 13, 33). The present studies have also revealed that β cell drug-induced desensitization is shared by glibenclamide and another structurally diverse antidiabetic drug with potential therapeutic importance, namely the guanidine derivative, BTS 67 582. The common and distinct pathways utilized by BTS 67 582 and efaroxan in relation to those of the sulfonylureas further highlight the complexity of this phenomenon.

In conclusion, further studies using clonal pancreatic β cells will help to elucidate the molecular mechanisms underlying drug-induced desensitization and K_{ATP} channel-dependent and K_{ATP} channel-independent pathways. Such knowledge should contribute significantly to understanding of β cell stimulus-secretion coupling

and identify novel therapeutic β cell targets for established and novel antidiabetic drugs.

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