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Specific desensitization of sulfonylurea- but not imidazoline-induced insulin release after prolonged tolbutamide exposure

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

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

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Abstract

Functional effects of prolonged exposure to the sulfonylurea, tolbutamide, were examined in the clonal electrofusion-derived BRIN-BD11 cell line. In acute 20-min incubations, 50–400 μM tolbutamide stimulated a dose-dependent increase ($P < 0.01$) in insulin release at both non-stimulatory (1.1 mM) and stimulatory (8.4 mM) glucose. Culture with 100 μM tolbutamide (18 hr) caused a marked (67%) decrease in subsequent insulin-secretory responsiveness to acute challenge with 200 μM tolbutamide, though notably, tolbutamide culture exerted no influence on 200 μM efaroxan-induced insulin secretion. Duration of exposure (3–18 hr) to 100 μM tolbutamide in culture also time-dependently influenced subsequent responsiveness to acute tolbutamide challenge, with progressive 47–58% decreases from 6–18 hr ($P < 0.001$). Similarly, 6- to 18-hr culture with 100 μM efaroxan specifically desensitized efaroxan-induced insulin release. Tolbutamide- and efaroxan-induced desensitization exhibited a time-dependent reversibility, with a sustained return to full insulin-secretory responsiveness by 12 hr. Notably, 18-hr culture with tolbutamide or efaroxan did not significantly affect insulinotropic responses to 16.7 mM glucose, 10 mM 2-ketoisocaproic acid, 10 mM alanine, 10 mM arginine, or 30 mM KCl. Diverse inhibitory actions of tolbutamide or efaroxan culture on late events in stimulus–secretion coupling reveal that drug desensitization is both a specific and important phenomenon. As such, the model system described could prove an important tool in determining the complex modes of action of established and novel clinically useful insulinotropic compounds.

Keywords: Sulfonylurea; Imidazoline; Desensitization; Clonal pancreatic beta cells; Insulin release

1. Introduction

Sulfonylureas have been one of the cornerstones of type 2 diabetes therapy for over 40 years, helping to counter the detrimental effects of insulin deficiency and progressive loss of pancreatic beta cell responsiveness associated with chronic hyperglycemia [1,2]. However, while the *in vivo* and *in vitro* insulinotropic actions of this important class of oral antidiabetic drug are undisputed, the precise mechanisms underlying the diverse effects of sulfonylureas on the pancreatic beta cell remain an important focus of current research [3–7].

Recent molecular and functional studies have established that the primary actions of sulfonylureas are exerted through high-affinity binding to the sulfonylurea receptor subunit (SUR1) of the beta cell K_{ATP} channel complex [8–11]. Sulfonylurea binding to the cell membrane K_{ATP} channel elicits a sequence of events including membrane depolarization and increased Ca^{2+} influx through voltage-dependent calcium channels (VDCC), ultimately resulting in insulin exocytosis [see 7,11,12 for reviews]. A number of other potentially useful oral insulinotropic agents, including efaroxan (an imidazoline), nateglinide (a phenylalanine derivative), repaglinide (a benzoic acid derivative), and BTS 67 582 (a guanidine derivative), are also believed to exert their primary actions through direct interaction with the K_{ATP} channel pore (Kir6.2) and/or SUR1 subunits [13–19]. Indeed, considerable interest has been generated regarding possible use of imidazolines and related compounds for type 2 diabetes therapy [16,20–24]. These compounds can act as potent insulinotropic agents and improve glucose homeostasis under conditions of glucose intolerance *in vivo* [21,22]. Such effects appear to be largely exerted through promoting insulin secretion as opposed to altering peripheral insulin sensitivity. The different actions of imidazolines from those

* Corresponding author. Tel.: +44-2870-324-781; fax: +44-2870-324-965.

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

Abbreviations: K_{ATP} channel, adenosine 5′ triphospate-sensitive potassium channel; KIC, 2-ketoisocaproic acid; PMA, phorbol 12-myristate 13-acetate; and SUR1, sulfonylurea receptor 1.

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of sulfonylureas constitute the basis for the development and exploitation of this new class of oral insulinotropic hypoglycemic agents [16,21–24].

While the detrimental chronic effects of hyperglycemia and hyperlipidemia on beta cell function remain a topic of much study [25–30], relatively little attention has been directed to other clinically important phenomena such as the progressive insensitivity to sulfonylureas in type 2 diabetes [1,31]. An increasing body of evidence suggests that the decline in glucose-lowering ability during long-term sulfonylurea therapy is attributable to a desensitization of the pancreatic beta cell to the actions of these drugs [32–34]. The phenomenon of desensitization is commonly observed in eukaryotic cells with an underlying role of cellular protection. It is largely attributed to changes in receptor-mediated cell-signaling events including modulation of gene expression, ion channels, protein phosphorylation, uncoupling from G proteins, and mitochondrial metabolism [35–42]. However, while desensitization is often defined as a temporary, readily induced, physiological, and reversible state of cellular refractoriness attributed to repeated or prolonged exposure to high concentrations of a stimuli, the mechanisms governing this phenomenon remain unclear.

Studies of the long-term effects of physiological and pharmacological agents on pancreatic beta cells are complicated by the phenotypic instability and relatively short functional lifespan of pancreatic islets *in vitro*. The recent emergence of stable cultured pancreatic beta cell lines with intact responses to glucose and other agents greatly facilitates research in this area [see 7,43,44]. The electrofusion-derived BRIN-BD11 cell line represents one such cellular model to examine the long-term effects of pharmacological agents on insulin secretion and pancreatic beta cell function. In this study, clonal BRIN-BD11 cells were utilized to examine the induction and reversibility of desensitization to sulfonylurea and imidazoline drugs. In addition to revealing important insights into the specificity of drug-induced desensitization and cellular targets, the present study highlights the potential of this approach to probe the diverse signaling mechanisms utilized by different classes of clinically relevant insulinotropic drugs.

2. Materials and methods

2.1. Chemicals

Reagents of analytical grade and deionized water (Purite) were used. RPMI-1640 tissue culture medium, fetal bovine serum, and antibiotics were from GIBCO, rat insulin standard was from Novo Nordisk, and ^{125}I-bovine insulin was from Lifescreen. All other chemicals were from Sigma and BDH Chemicals.

2.2. Cell culture

Clonal pancreatic BRIN-BD11 cells (passage 28–35) were used for this study. Characteristics of this electrofusion-derived glucose-responsive cell line have been described elsewhere [45–50]. BRIN-BD11 cells typically retain their functional features after long-term culture for up to 50 passages, thus offering an attractive alternative to cultured pancreatic beta cells, which exhibit a relatively short functional lifespan.

BRIN-BD11 cells were grown in RPMI-1640 tissue culture medium containing 11.1 mM glucose and 0.3 g/L of L-glutamine, and supplemented with 10% (v/v) fetal bovine serum, 100 IU/mL of penicillin, and 0.1 g/L of streptomycin at 37° with 5% CO_2 and 95% air. Cells were washed with Hanks' balanced saline solution (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 (3–18 hr) in the absence (standard culture conditions) or presence of either 100 μM tolbutamide or 100 μM efaroxan at 37°. Culture medium was then replaced with 1 mL of a Krebs–Ringer bicarbonate (KRB) buffer consisting of (in mM) 115 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.28 $CaCl_2$, 1.2 KH_2PO_4, 25 HEPES, and 8.4% (w/v) $NaHCO_3$ (pH 7.4) supplemented with 0.05% BSA and 1.1 mM glucose [45,46]. After 40-min preincubation at 37°, the buffer was replaced with 1 mL of KRB test buffer containing either 1.1 or 8.4 mM glucose, in the presence or absence of test agents as detailed in the legends to figures. After 20-min incubation at 37°, aliquots of test buffer were removed and stored at −20° for insulin radioimmunoassay [45].

2.3. Statistical analyses

Results are expressed as means ± SE of six independent observations. Groups of data were compared using unpaired Student's *t*-test and differences considered significant if $P < 0.05$.

3. Results

3.1. Insulinotropic responses at non-stimulatory and stimulatory glucose concentrations

Tolbutamide (50–400 μM) evoked a stepwise 1.2- to 2.1-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) concentrations of glucose (Fig. 1A). Efaroxan showed a similar pattern of insulin-secretory responsiveness, with 50–400 μM evoking respective 1.5- to 2.9-fold ($P < 0.001$) and 1.2- to 2.1-fold ($P < 0.05$ to $P < 0.001$) increases at 1.1 and 8.4 mM glucose (Fig. 1B). Characteristic of the BRIN-BD11 cells [45,46,48], raising

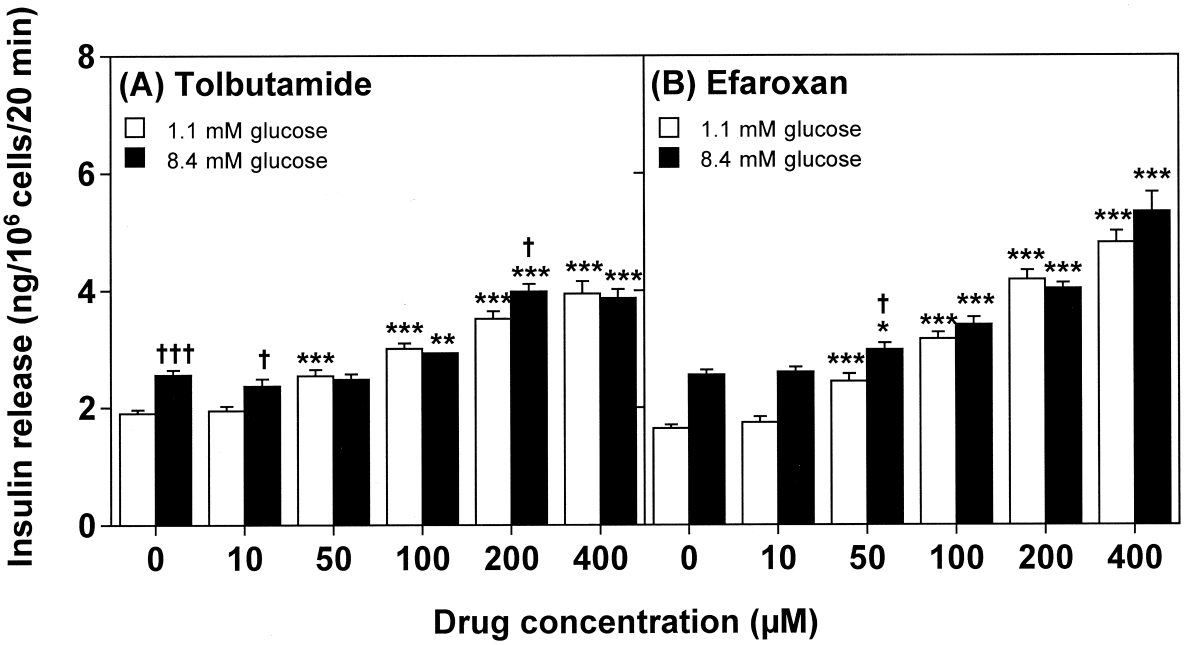

Fig 1. Effects of 0–400 μM of either tolbutamide (A) or efaroxan (B) at non-stimulatory (1.1 mM) or stimulatory (8.4 mM) glucose. Following 40 min of preincubation with a buffer containing 1.1 mM glucose, effects of either tolbutamide or efaroxan were tested during a 20-min incubation period. Values 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 or efaroxan. $^{\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.001$ compared with respective effects at 1.1 mM glucose.

the glucose concentration from 1.1 to 8.4 mM stimulated a 1.4-fold ($P < 0.001$) insulin-secretory response (Fig. 1).

3.2. Responses to insulinotropic drugs following culture with tolbutamide or efaroxan

As shown in Fig. 2, 200 μM of tolbutamide or efaroxan elicited respective 2.1- and 2.6-fold ($P < 0.001$) insulin-secretory responses after 18-hr exposure to normal culture conditions. However, 18-hr culture with 100 μM tolbutamide markedly reduced (by 67%, $P < 0.001$) the subsequent insulin-releasing action of acute tolbutamide exposure (Fig. 2A). Notably, 18-hr culture with 100 μM tolbutamide did not affect subsequent responsiveness to efaroxan (Fig. 2A), indicating distinct sites of action of these two different classes of drugs. After 18-hr culture with 100 μM efaroxan, a similar pattern emerged, with a 53% reduction ($P < 0.001$) in the subsequent insulin-secretory effects of efaroxan compared with normal culture conditions, whilst the insulinotropic actions of tolbutamide remained intact (Fig. 2B).

3.3. Time course of desensitization of drug-induced insulinotropic actions

Subsequent responses to tolbutamide and efaroxan were examined following 3, 6, 12, and 18 hr culture with tolbutamide to examine the time–course of desensitization (Fig. 3). As shown in Fig. 3A, there was a notable decrease (44%, $P < 0.01$) in the secretory effects of tolbutamide following 3-hr tolbutamide culture, with a progressive (47–58%, $P < 0.001$) decline in secretory response after 6–18 hr. Efaroxan-induced insulin release was not affected at any time following 3- to 18-hr culture with tolbutamide (Fig. 3B). Conversely, whereas exposure (3, 6, 12, or 18 hr) to 100 μM efaroxan in culture exerted no effect on tolbutamide-induced insulin release (Fig. 4A), these conditions progressively lowered subsequent responsiveness to efaroxan (Fig. 4B). Efaroxan culture desensitized subsequent responsiveness to the imidazoline following a similar pattern to tolbutamide desensitization, reaching significance at 6 hr (36% decrease, $P < 0.01$) with maximum suppression (by 57%, $P < 0.001$) by 18 hr (Fig. 4B). It is important to note that in each instance, cell number, basal insulin secretion (at 1.1 mM glucose), and cell viability (assessed using trypan blue) were unaffected by the culture conditions. Equally important, cellular insulin content was remarkably constant under the culture conditions employed, remaining in the range 64–71 ng/10^6 cells.

3.4. Reversibility of drug-induced desensitization of insulinotropic actions

Recovery of the insulinotropic capacities of tolbutamide or efaroxan in culture were assessed after recovery periods of 6 and 12 hr. The return of cells to normal culture conditions following 18-hr culture with 100 μM tolbutamide resulted in a 256% increase ($P < 0.001$) in insulin-secretory responsiveness to tolbutamide by 6 hr, with a complete recovery by 18 hr (Fig. 5A). Similarly, after 18-hr exposure

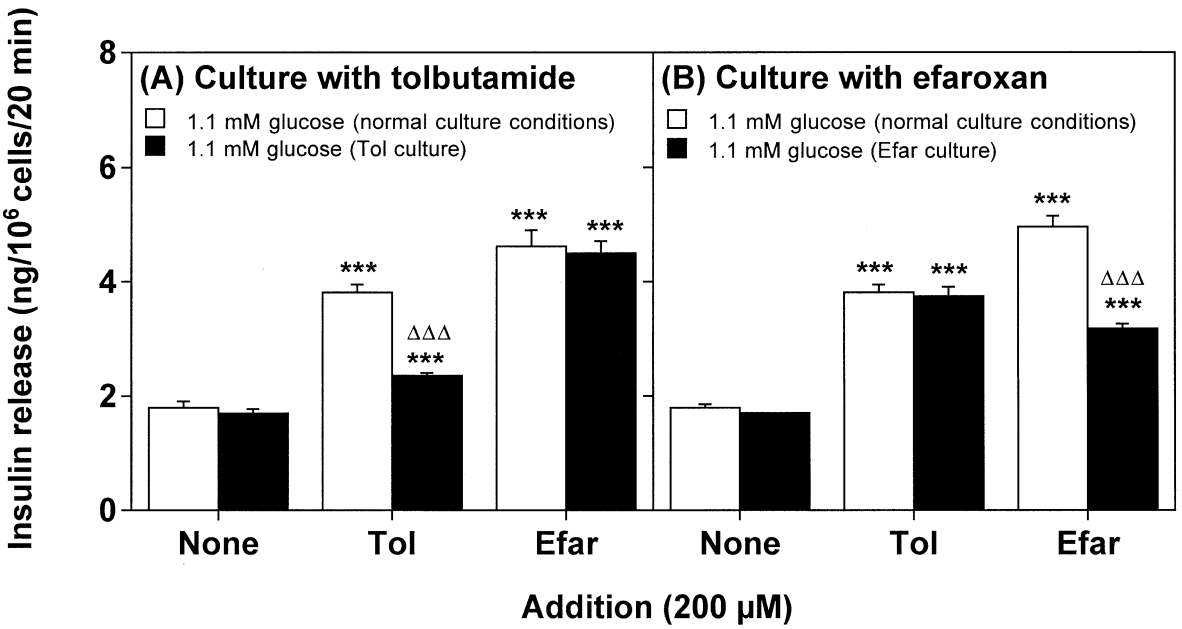

Fig 2. Effects of culture with tolbutamide (A) or efaroxan (B) on sulfonylurea- and imidazoline-induced insulin secretion. After 18-hr culture in the absence (normal culture conditions) or presence of either 100 μM tolbutamide (Tol culture) or 100 μM efaroxan (Efar culture), cells were preincubated for 40 min before 20-min acute incubation with a buffer containing 1.1 mM glucose in the absence or presence of either 200 μM tolbutamide (Tol) or 200 μM efaroxan (Efar). Values are means ± SEM for 6 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 normal culture conditions.

to efaroxan, the return to normal culture conditions resulted in a significant 147% increase ($P < 0.001$) in the insulin response to efaroxan after 6 hr, representing a sustained return to full insulin-secretory responsiveness (Fig. 5B). Basal insulin secretion (at 1.1 mM glucose) was not affected by the culture conditions (Fig. 5), and insulin content remained in the normal range (data not shown). Additional studies were performed to examine whether 40-min expo-

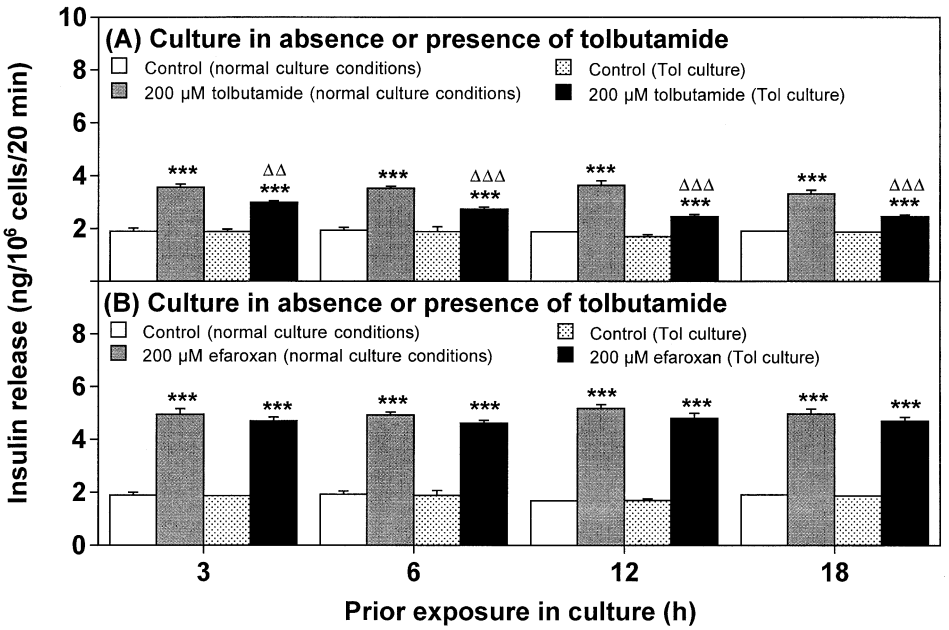

Fig 3. Time-dependent effects of culture with tolbutamide on subsequent insulin-secretory responsiveness to tolbutamide (A) and efaroxan (B). After 3, 6, 12, or 18 hr culture in the absence (normal culture conditions) or presence of 100 μM tolbutamide (Tol culture), cells were preincubated for 40 min before 20-min acute incubation with a buffer containing 1.1 mM glucose in the absence or presence of either 200 μM tolbutamide (Tol) or 200 μM efaroxan. Values are means ± SEM for 6 separate observations. ***$P < 0.001$ compared with respective effects of control (1.1 mM glucose); $^{\Delta\Delta}P < 0.001$; $^{\Delta\Delta\Delta}P < 0.001$ compared with respective effects after normal culture conditions.

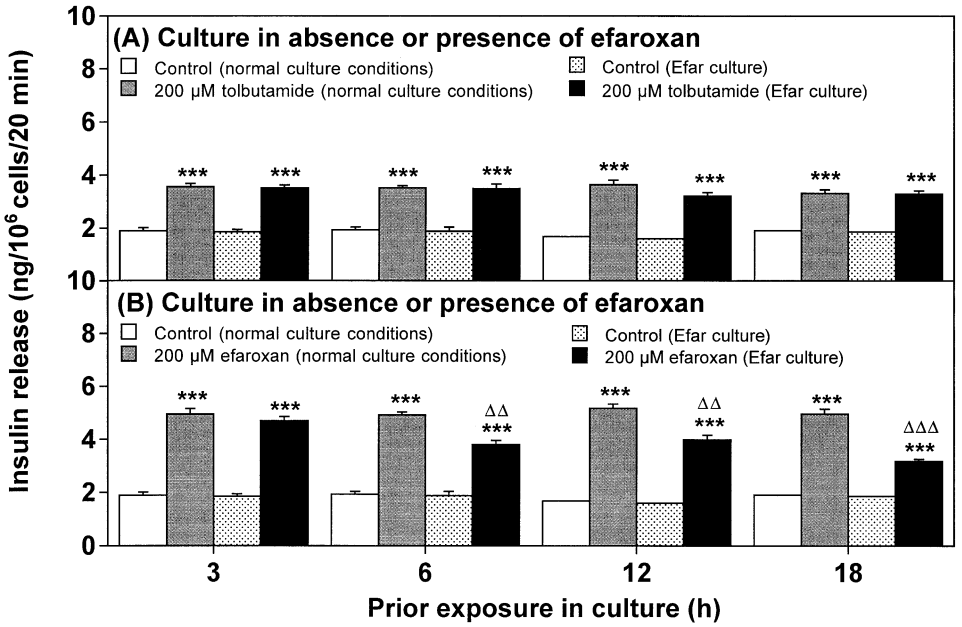

Fig 4. Time-dependent effects of culture with efaroxan on subsequent insulin-secretory responsiveness to tolbutamide (A) and efaroxan (B). After 3, 6, 12, or 18 hr culture in the absence (normal culture conditions) or presence of 100 μM efaroxan (Efar culture), cells were preincubated for 40 min before 20-min acute incubation with a buffer containing 1.1 mM glucose in the absence or presence of either 200 μM tolbutamide or 200 μM efaroxan (Efar). Values are means ± SEM for 6 separate observations. ***$P < 0.001$ compared with respective effects of control (1.1 mM glucose). $^{\Delta\Delta}P < 0.01$, $^{\Delta\Delta\Delta}P < 0.001$ compared with respective effects after normal culture conditions.

sure to preincubation buffer in the absence of either tolbutamide or efaroxan was sufficient to allow a partial reversal of the desensitization. Notably, after 18-hr culture with either tolbutamide or efaroxan, drug-induced insulin output was equally blunted, irrespective of preincubation conditions (data not shown).

3.5. Actions of drug-induced desensitization on responses to other insulin secretagogues

Insulin responses to glucose, a range of keto and amino acids, depolarizing concentrations of KCl, cholinergic stimulation, and activators of adenylate cyclase and protein kinase C were examined after 18-hr culture in the absence or presence of 100 μM tolbutamide or 100 μM efaroxan. As shown in Fig. 6, raising the glucose concentration from 1.1 to 16.7 mM stimulated a 1.7-fold increase ($P < 0.001$) in insulin secretion, which was unaffected by tolbutamide or efaroxan culture. Similarly, responses to the 2-keto acid, KIC (2-fold increase; $P < 0.001$), the metabolizable amino acid, alanine (4.9-fold; $P < 0.001$), arginine (2.7-fold increase; $P < 0.001$), and 30 mM KCl (7.3-fold increase; $P < 0.001$) were unaffected by tolbutamide or efaroxan culture (Fig. 6). As shown in Fig. 7, the 1.2-fold increase ($P < 0.01$ to $P < 0.001$) in insulin secretion induced by the cholinergic receptor agonist, carbachol, was also totally unaffected by tolbutamide or efaroxan culture. However, culture with tolbutamide resulted in a 25% reduction ($P < 0.001$) in the secretory responsiveness to the adenylate cyclase activator, forskolin. Notably, efaroxan culture, while not affecting forskolin-induced insulin secretion, resulted in a modest but significant ($P < 0.05$) 12% increase in the secretory actions of PMA, a known activator of protein kinase C (PKC) (Fig. 7).

4. Discussion

The present study examines the phenomenon of drug desensitization in the pancreatic beta cell using the clonal glucose-responsive BRIN-BD11 cell line [45]. Functional consequences of prolonged exposure to the insulinotropic antidiabetic drugs tolbutamide and efaroxan were examined. Both classes of drug are known to primarily act through the K_{ATP} channel complex [8–11,14,15,51,52], and thus particular emphasis was directed to establishing the long-term effects of these agents on responses to physiological and pharmacological regulators of K_{ATP} channel activity and membrane depolarization.

Consistent with previous observations, tolbutamide and efaroxan served as both initiators and potentiators of insulin release in BRIN-BD11 cells [7,18,52]. In the presence of non-stimulatory (1.1 mM) or sub-maximal stimulatory (8.4 mM) glucose concentrations, both drugs elicited dose-dependent insulin-secretory responses with maximal effects at 200–400 μM. In accordance with the hypothesis that prolonged exposure to sulfonylureas desensitizes the pancreatic beta cells [31–34], 18-hr culture with tolbutamide markedly decreased the subsequent acute insulin-releasing effects of this agent. Interestingly, however, the insulinotropic re-

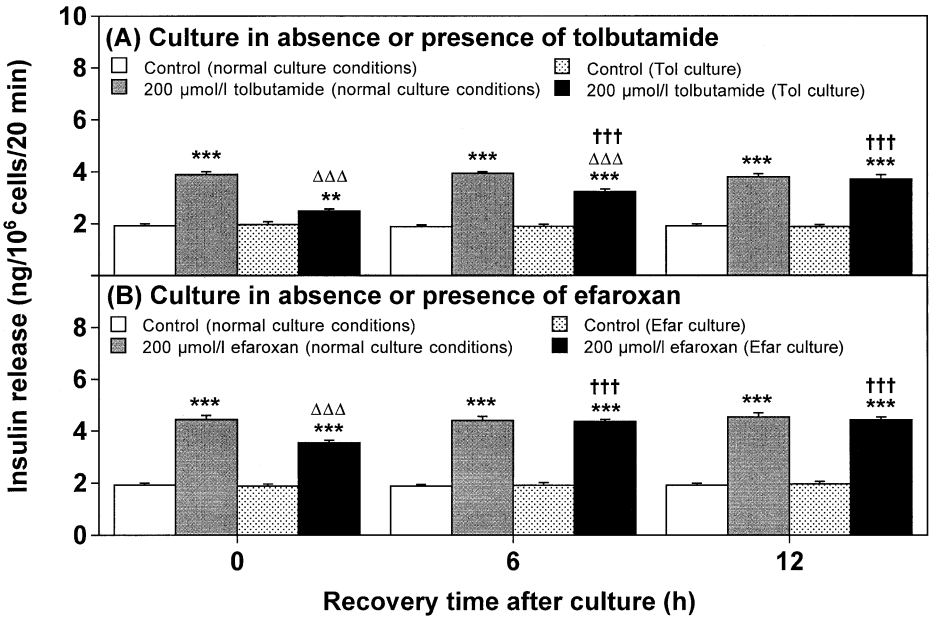

Fig 5. Time-dependent recovery of insulin-secretory responsiveness to tolbutamide (A) or efaroxan (B) after 18-hr culture with either tolbutamide (A) or efaroxan (B). After 18-hr culture in the absence (normal culture conditions) or presence of either 100 μM tolbutamide (Tol culture) or 100 μM efaroxan (Efar culture), cells were cultured for a further 0, 12, or 18 hr in the absence of drug. This was followed by preincubation (40 min) and subsequent 20-min incubation with a buffer containing 1.1 mM glucose in the absence or presence of either 200 μM tolbutamide or 200 μM efaroxan. Values are means ± SEM for 6 separate observations. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with respective effects of control (1.1 mM glucose). $^{\Delta\Delta\Delta}P < 0.001$ compared with respective effects after normal culture conditions. $^{\dagger\dagger\dagger}P < 0.001$ compared with respective effects at 0 hr.

sponse to efaroxan remained intact following tolbutamide culture. Conversely, culture with efaroxan for 18 hr, while exerting no effect on the tolbutamide-induced insulin output, significantly diminished the insulinotropic activity of efaroxan. The specific nature of the desensitization with each of these agents is important in that it reveals differential modes of action of these two distinct types of drug. In addition, it implies that desensitization does not adversely affect the general functionality of the K_{ATP} channel, through which both agents are ultimately believed to exert their insulinotropic effects [3,11,51,52]. Taken together, these observations provide evidence for diverse binding sites through which sulfonylureas and efaroxan exert their actions on the beta cell. While the effects of sulfonylureas are believed to primarily act though binding SUR1 [8–11], imidazoline drugs are currently thought to influence K_{ATP} channel activity both by direct interaction with Kir6.2 and through a putative imidazoline receptor at a site distal to the channel [10,11,15,53,54].

Evaluation of the time–course of desensitization to tolbutamide and efaroxan revealed a significant decrease in insulin output after 3-hr exposure to tolbutamide, but after 6-hr culture with efaroxan. Desensitization to the acute actions of both drugs increased steadily to a maximum inhibition after 18 hr. Such effects were readily reversed by subsequent culture for 6 to 12 hr in the absence of tolbutamide or efaroxan. Complete restoration of the efaroxan response was achieved within 6 hr, whereas 12-hr culture was required to fully reverse desensitization to tolbutamide. The different kinetics suggest respective differences in access and binding to functional sulfonylurea and imidazoline sites in the pancreatic beta cell. The specificity of desensitization to the drug used in culture, together with the nature of reversibility, clearly argues against cell toxicity as a simple explanation for these observations. In addition, desensitization induced by tolbutamide or efaroxan was independent of changes in cell number, basal insulin secretion, cellular insulin content, or cell viability. When considering the actions of tolbutamide, it is interesting to note that the acute effects of sulfonylureas on beta cells involve both stimulatory and inhibitory components [55,56]. Taken together, this provides evidence for specific targeting of drug-mediated signaling pathways in the present study as opposed to general alterations of cell growth, survival, or function.

Additional experiments were performed to assess whether drug desensitization impaired the actions of any other important regulators of pancreatic beta cell function. After normal culture conditions, acute challenge with KIC, alanine, arginine, or a depolarizing concentration of KCl evoked characteristic increases in insulin secretion from the BRIN-BD11 cells [7,45,47,49]. Prior culture with tolbutamide or efaroxan did not affect the insulinotropic actions of any of these agents. These data demonstrate that physiological stimulus–secretion coupling pathways are generally intact under conditions of drug desensitization, indicating that tolbutamide and efaroxan have both specific and distinct modes of action on the beta cell. The clear-cut

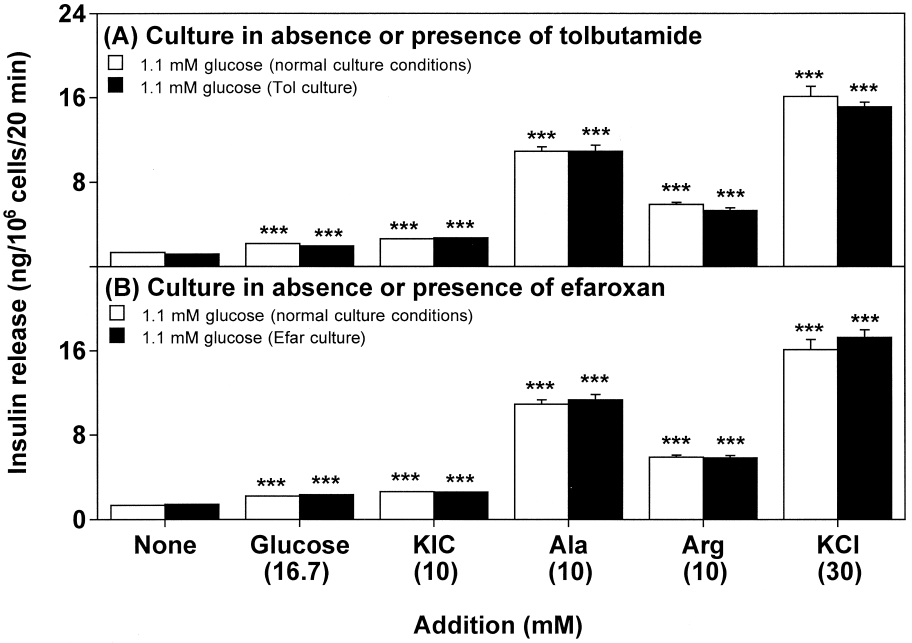

Fig 6. Effects of culture with tolbutamide (A) or efaroxan (B) on insulin-secretory responses to agents acting through metabolism and alterations of membrane potential. After 18-hr culture in the absence (normal culture conditions) or presence of 100 μM tolbutamide (Tol culture, A) or 100 μM efaroxan (Efar culture, B), 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 the addition of 15.6 mM glucose (final concentration 16.7 mM), 10 mM KIC, 10 mM L-alanine (Ala), 10 mM L-arginine (Arg), or 30 mM KCl. Values are means ± SEM for 6 separate observations. ***$P < 0.001$ compared with respective effects in the absence of addition.

stimulatory actions of these nutrient secretagogues also argue against an important role of beta cell hyperactivity [57] in drug-induced desensitization. Intact functional responses also indicate that GLUT-2, glucokinase, K_{ATP} channels, voltage-dependent Ca^{2+} channels, and other similar structures are not major sites involved in drug desensitization [7,12,52]. However, examination of protein kinase A (PKA)-mediated (forskolin) and protein kinase C (PKC)-mediated (PMA) signaling pathways indicated that drug desensitization cannot be solely attributed to a specific desensitization of the sulfonylurea or imidazoline receptors. The impairment of forskolin-induced insulin release by tolbutamide culture and the potentiation of PMA action following efaroxan culture suggest involvement of complex receptor-mediated pathways which may be linked to late events governing insulin exocytosis.

Collectively, these data reveal that while sulfonylurea and imidazoline drugs may both primarily act through the K_{ATP} channel, the specific desensitization of their insulinotropic actions cannot be directly attributed to a general impairment of K_{ATP} channel function. While desensitization of SUR1 or indeed a distal imidazoline receptor may not render the K_{ATP} channel dysfunctional, it appears unlikely that receptor sequestration can adequately explain the desensitization phenomenon. Previous studies have shown that although the ATP-inhibitory site lies on Kir6.2 [15], the presence of SUR1 substantially enhances the sensitivity of Kir6.2 to ATP, and imidazolines may exert direct actions on Kir6.2 [58,59]. Applying these observations to the present data, several possible outcomes can be hypothesized. Firstly, if tolbutamide desensitization resulted in a down-regulation or desensitization of SUR1, the secretory responses attributed to ATP generation from glucose and KIC should be blunted after tolbutamide culture. Similarly, if efaroxan culture resulted in the sequestration of imidazoline-binding sites, then a down-regulation or desensitization of Kir6.2 may be expected. Both these hypothetical outcomes appear largely inconsistent with the intact responses to nutrient regulators of K_{ATP} channel function after prolonged exposure to tolbutamide or efaroxan. From the current data, it appears more likely that a proximal signal defect for closure of the K_{ATP} channels arises from tolbutamide or efaroxan desensitization, rather than an intrinsic defect in the channel. More intriguingly, elevation of the intracellular calcium concentration ($[Ca^{2+}]_i$) by efaroxan appears to be intact under conditions of efaroxan desensitization, and beta cell SUR1 expression is increased [52]. Unraveling the mechanisms underlying these observations seems likely to greatly increase understanding of complex interplay between factors controlling exocytosis.

In summary, the present study has shown that more than 3- to 6-hr exposure to tolbutamide or efaroxan in culture may result in a specific and readily reversible desensitization to insulinotropic drug action. Elucidation of underlying molecular mechanisms may contribute significantly to the understanding of beta cell stimulus–secretion coupling and targets of antidiabetic drugs. Desensitization to drug action may have therapeutic implications [1,31–34]. The present data suggest that sulfonylureas and imidazolines, represented by tolbutamide and efaroxan, seem to be equipotent

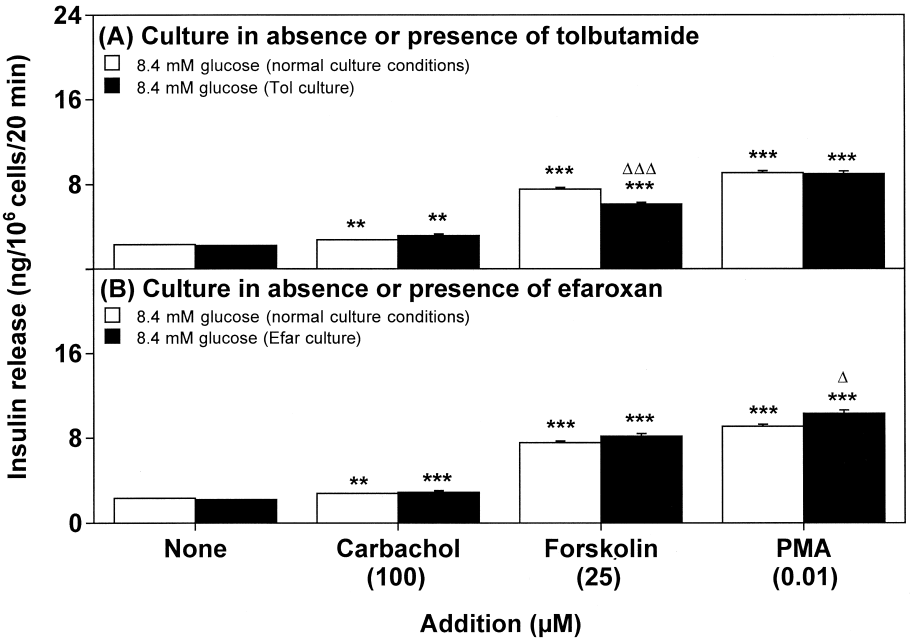

Fig 7. Effects of culture with tolbutamide (A) or efaroxan (B) on insulin-secretory responses to carbachol, forskolin, or PMA. After 18-hr culture in the absence (normal culture conditions) or presence of 100 μM tolbutamide (Tol culture, A) or 100 μM efaroxan (Efar culture, B), cells were preincubated for 40 min before 20-min acute incubation with a buffer containing 8.4 mM glucose in the absence or presence of 100 μM carbachol, 25 μM forskolin, or 10 nM PMA. Values are means ± SEM for 6 separate observations. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with respective effects in the absence of addition. $^{\Delta}P < 0.05$, $^{\Delta\Delta\Delta}P < 0.001$ compared with respective effects after normal culture conditions.

in inducing drug desensitization. Possible advantages of one drug over another will therefore be related to lower therapeutic concentration and shorter half-life. The model system described here could prove an important tool in ongoing research aiming to unravel the complex modes of actions of drugs acting through the K_{ATP} channel, and in the discrimination and isolation of cellular binding sites utilized by established and novel clinically useful insulinotropic compounds. Further clinical studies are warranted to assess the implications of these findings and to determine the optimum long-term administrative regimens for these important orally effective antidiabetic drugs.

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