Pflügers Arch – Eur J Physiol (1996) 432 : 89–96

ORIGINAL ARTICLE

Kirsten-Berit Abel · Sabine Lehr · Susanne Ullrich

Adrenaline-, not somatostatin-induced hyperpolarization is accompanied by a sustained inhibition of insulin secretion in INS-1 cells. Activation of sulphonylurea K^+_{ATP} channels is not involved

Received: 10 October 1995/Received after revision: 3 January 1996/Accepted: 9 January 1996

Abstract Adrenaline and somatostatin inhibit insulin secretion via pertussis toxin (PTX)-sensitive mechanisms. Since glucose-stimulated release involves inhibition of ATP-sensitive K^+ (K^+_{ATP}) channels and activation of Ca^{2+} influx, we took advantage of the glucose-sensitive, insulin-secreting cell line INS-1 to investigate whether inhibitors of insulin release modulate membrane voltage and K^+_{ATP} channel activity in cell-attached patch-clamp experiments. We found that adrenaline, through α_2-adrenoceptors, and somatostatin counteracted glucose-induced depolarization and action potentials. As expected, these effects were mediated via PTX-sensitive G proteins since PTX pretreatment of the cells eliminated the effects of adrenaline and somatostatin on membrane voltage. When INS-1 cells were activated by adding both the K^+_{ATP} channel inhibitor tolbutamide and the adenylyl cyclase activator forskolin, adrenaline and somatostatin still repolarized the plasma membrane. Single-channel measurements in the cell-attached mode revealed that tolbutamide closed a 40 to 70 pS K^+ channel which was neither reopened by adrenaline nor by somatostatin. In parallel cell preparations, insulin secretion was measured by radioimmunoassay. Insulin release induced by glucose, forskolin and tolbutamide was abolished by adrenaline. In contrast, somatostatin attenuated insulin secretion by only 30%. After comparing the potency of adrenaline and somatostatin on membrane voltage and on insulin secretion, it is concluded that the repolarizing effect of adrenaline on membrane voltage is not sufficient to explain its potent inhibitory effect on insulin secretion.

K-B. Abel · S. Lehr · S. Ullrich (✉)
Physiologisches Institut der Albert-Ludwigs-Universität, Hermann-Herder-Strasse 7, D-79104 Freiburg, Germany

Key words Insulin secretion · Adrenaline · α_2-Adrenoceptors · Somatostatin · Patch-clamp method · β-cell membrane voltage · Sulphonylurea-sensitive K^+_{ATP} channels

Introduction

A major defect in NIDDM (non-insulin-dependent diabetes mellitus) is the dysfunction of insulin-secreting β-cells which do not secrete sufficient insulin to lower elevated blood glucose concentrations [22]. Thus, our understanding of the regulation of insulin secretion is crucial for effective treatment of the disease. Some intracellular events involved in islet cell function are already clarified. Hence, augmented glucose entry into the cells increases both the metabolic flux and the generation of ATP [21]. It is now generally accepted that the link between enhanced glucose metabolism and Ca^{2+} influx, which is necessary for the induction of insulin secretion, is the ATP-mediated inhibition of K^+ channels [4, 7]. These channels are also the targets of sulphonylureas [26], which are hypoglycaemic drugs used for the treatment of NIDDM. Recently, a sulphonylurea-binding protein has been cloned and this may be a modulatory protein of K^+ channel activity [1]. The β-cell depolarization resulting from closure of ATP-sensitive K^+ (K^+_{ATP}) channels leads to the opening of L-type Ca^{2+} channels and to Ca^{2+} influx [5, 10, 21].

A variety of substances are known to inhibit the stimulatory effects of glucose and sulphonylureas. Among these are adrenaline and noradrenaline, somatostatin and galanin [2, 13, 18, 29–32]. These substances are known to bind to specific membrane receptors which are coupled to pertussis-toxin-sensitive G proteins [14] modifying a variety of cellular parameters involved in signal transduction. Thus, activation of these receptors inhibits adenylyl cyclase and lowers adenosine 3′,5′-cyclic monophosphate (cAMP) levels

[2, 14]. Moreover, α_2-adrenoceptor activation, as well as somatostatin and galanin have been shown to inhibit Ca^{2+} channels [12, 27] and to repolarize the plasma membrane [8, 19, 25]. In addition, experiments performed using permeabilized cells, in which membrane channels are bypassed and soluble cytosolic messengers such as Ca^{2+} are determined by the concentration in the bath solution, showed that inhibitors of insulin secretion are still functioning, which implies direct effects of these modulators on insulin secretion [30–32]. Recently, G_i proteins have been located on insulin-secreting granule membranes [16].

The involvement of the described effects to elicit inhibition of insulin secretion under physiological conditions is not completely understood. Experimental evidence suggests that inhibition of adenylyl cyclase and the effects on ion channels are not sufficient to explain the inhibition of secretion. Thus, in the past, we and others were able to show that insulin secretion is still abolished by adrenaline in the presence of elevated cAMP levels [18, 29]. The effects on membrane voltage were transient and could be dissociated from inhibition of insulin release [8]. Moreover, the interpretation became even more complicated, because results obtained with insulin-secreting cell lines differed from data obtained with normal β-cells. Thus, in RINm5F and HIT cells, activation of K^+_{ATP} channels by somatostatin and galanin leading to hyperpolarization was observed [9, 23, 33, 34], whereas in normal β-cells a small K^+ channel distinct to the K^+_{ATP} channels was activated by the α_2-adrenoceptor agonist clonidine [24].

In this study we used the INS-1 cells, a glucose-sensitive insulin-secreting cell line [3], which has been proven to be much less dedifferentiated than other insulin-secreting cell lines such as RINm5F and HIT cells, to evaluate the effects of inhibitory hormones on membrane voltage, K^+ channel activity and insulin secretion. It was found that the repolarizing effect of adrenaline does not involve K^+_{ATP} channels and is not sufficient to account for the potent inhibitory effect of insulin secretion.

Materials and methods

Cell culture

INS-1 cells culture was performed as previously described [3]. Briefly, cells were treated with trypsin and seeded every week at a cell density of 250×10^6 cells/l in 20 ml of RPMI 1640 culture medium (GIBCO, Eggenstein, Germany) supplemented with 50 µmol/l 2-mercaptoethanol, 1 mmol/l sodium pyruvate, 10 mmol/l 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 100 000 IU/l penicillin, 100 mg/l streptomycin and 100 g/l decomplemented fetal calf serum (Seromed, Berlin, Germany) into 75 cm^2 culture flasks. The medium was changed twice per week. All substances used were purchased from Sigma (Munich, Germany) or RBI, Bio Trend (Cologne, Germany).

Patch-clamp measurements

Patch-clamp measurements were performed using cells (0.2×10^6 cells, passages 80–100) seeded on glass coverlips coated with poly-L-ornithine (10 mg/l, Sigma). After 2–5 days of culture in standard culture medium the coverslip was mounted onto the stage of an inverted microscope (Axiovert 10, Zeiss, Germany) and perifused continuously at 37° C with a solution containing (in mmol/l): 140 NaCl, 5.6 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 0.5 glucose and 10 HEPES, pH 7.4. A flowing KCl (1 mol/l) electrode served as a reference and appropriate corrections for liquid junction voltages were made. The patch-clamp pipettes (Clark-Medical, Reading, UK) with an input resistance of 5–8 MΩ were pulled automatically (DMZ Universal Puller, Zeitz, Augsburg, Germany) and coated with bee's wax. The pipette solution contained (in mmol/l): 130 KCl, 4 $MgCl_2$, 2 $CaCl_2$, 10 ethylenebis(oxonitrilo)tetraacetate (EGTA), 0.65 Na_2ATP and 20 HEPES, pH 7.15. After seal resistances of ≥ 1 GΩ were obtained, membrane voltage (V_m) was measured continuously using the current-clamp mode of the patch-clamp amplifier (U. Fröbe, and R. Busche, this institute, Freiburg, Germany) and was displayed by a pen recorder. The pipette capacitance was cancelled by the compensation circuit of the amplifier (U. Fröbe and R. Busche, this institute) using a sine wave command voltage [15]. Single-channel measurements were performed in the voltage-clamp mode and current/voltage (I/V) curves were obtained by clamping the pipette voltage to ± 80 mV. The data was low-pass filtered (3 kHz) and stored on digital audio tape. Patch-clamp software written by U. Fröbe (this institute) was used for channel analysis.

Insulin secretion

Insulin secretion was measured in cells (0.25×10^6 cells per well) seeded into 24-hole multiwell dishes. After 2 days of culture, the cells were washed with a modified KRB-HEPES buffer containing (in mmol/l): 136 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1 $CaCl_2$, 1.2 KH_2PO_4, 5 $NaHCO_3$, 0.5 glucose, 10 HEPES, pH 7.4 and 0.5% bovine serum albumin (fraction V, Sigma). They were preincubated at 37° C for 30 min in the same buffer and incubated for another 30 min in a buffer containing the test substances at the concentrations indicated. Insulin released into the supernatant and insulin content of the cells after acid ethanol extraction was measured by a radioimmunoassay as described previously [11] utilizing rat insulin (Novo Nordisk, Bagsvaerd, Denmark) as standard and rabbit anti-insulin porcine antiserum (Calbiochem). ^{125}I-labelled insulin was from CIS Diagnostik (Dreieich, Germany).

Statistical analysis

Data are presented as mean ± SEM. Student's t-tests for paired data of patch-clamp experiments and for unpaired data of insulin secretion experiments were used for statistical analysis ($P < 0.05$).

Results

Effects of adrenaline and somatostatin on membrane voltage in INS-1 cells

In cell-attached measurements, adrenaline and somatostatin abolished action potentials induced by glucose and hyperpolarized the plasma membrane (Fig. 1A, C). At 1 µmol/l adrenaline, the effect was persistent, but reversible after removal of the catecholamine. At lower

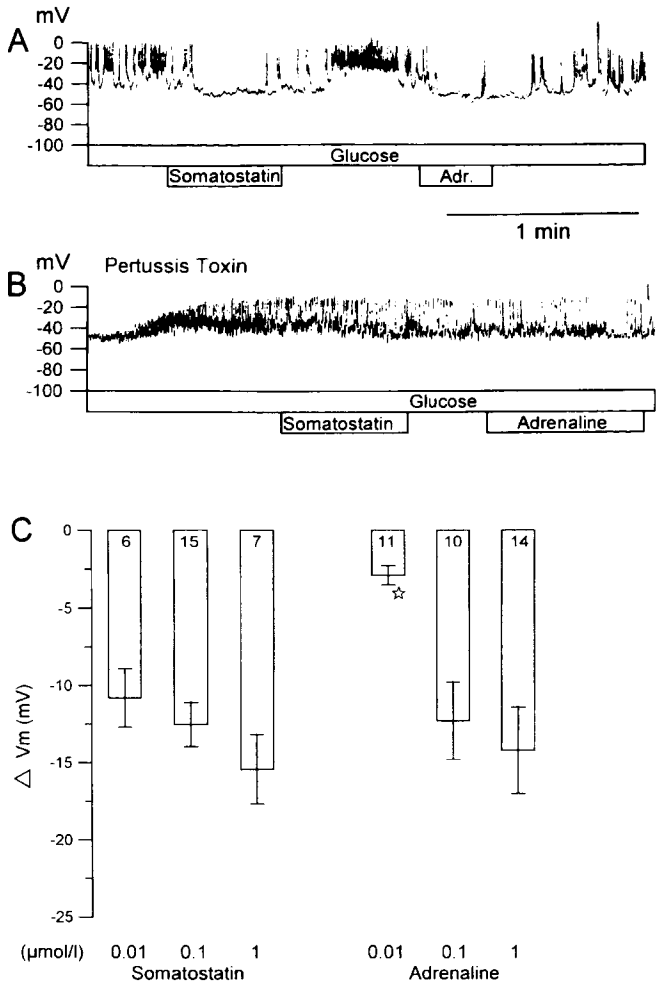

Fig. 1A–C Effects of adrenaline and somatostatin on membrane voltage in INS-1 cells. Measurements of membrane voltage were performed as described in the Materials and methods. **A–C** Depolarization and action potentials were induced by glucose (15 mmol/l). **A** Control cells were superfused with somatostatin and adrenaline (0.1 μmol/l each) as indicated. **B** Cells were incubated for 3 h with 100 μg/l pertussis toxin added to the culture medium prior to the patch-clamp experiment. **C** Maximal hyperpolarizations induced by increasing concentrations of somatostatin and adrenaline plotted as mean ± SEM for the indicated number of observations as shown in each *column* (*significant vs the values prior to addition)

concentrations action potentials also reappeared in the continuous presence of adrenaline. Somatostatin proved to be as potent as adrenaline, repolarizing the plasma membrane at 0.1 μmol/l by 13 mV.

Pertussis toxin pretreatment of the cells abolished the effects of adrenaline and somatostatin ($n = 11$, Fig. 1B). The treatment itself did not alter either basal membrane voltage (-64 ± 4 mV and -57 ± 6 mV, $n = 9$, in treated and control cells, respectively) or the responsiveness of the cells to glucose (data not shown).

Two α_2-adrenergic agonists, clonidine, 1 μmol/l, and UK14, 304, 1 μmol/l, mimicked the adrenaline-evoked effect (Fig. 2B and C, respectively). Rauwolscine (10 μmol/l), an α_2-adrenergic antagonist, counteracted the adrenaline-induced hyperpolarization whereas prazosin (10 μmol/l) an α_1-adrenergic antagonist, and propranolol (10 μmol/l), a β-adrenergic antagonist, had no effects on membrane voltage in the presence of adrenaline in INS-1 cells (Fig. 2D–F).

These results indicate that somatostatin and adrenaline, the latter through activation of α_2-adrenoceptors, hyperpolarize INS-1 cell plasma membrane via pertussis-toxin-sensitive G protein(s).

In an additional series of experiments, adrenaline and somatostatin were tested in the presence of the sulphonylurea tolbutamide, 50 μmol/l, and forskolin, 5 μmol/l, a stimulator of adenylyl cyclase. This combination of stimulators was proven to potently increase insulin release (Fig. 6, Table 1 and S. Ullrich et al. unpublished observations). As can be seen in Fig. 3, adrenaline and somatostatin significantly repolarized the plasma membrane even in the presence of these stimulators.

To investigate whether the adrenaline-evoked effect on membrane voltage is accompanied by a change in whole-cell conductance, we performed experiments using the amphotericin B-perforated patch technique to gain electrical access to the cell cytosol [5]. To this end the pipette was backfilled with a solution supplemented with 250 mg/l amphotericin B. The resting membrane voltage was -73 ± 2.7 mV ($n = 9$). Glucose (15 mmol/l) depolarized the cells to -56 ± 2.4 mV ($n = 9$) and induced action potentials. Subsequent addition of 1 μmol/l adrenaline in the presence of glucose repolarized the cells to -63.4 ± 2.2 mV ($n = 9$). In the voltage-clamp mode ± 10 mV steps were performed for current measurements. The basal whole-cell

Table 1 Effects of adrenergic agonists and antagonists on adrenaline-evoked inhibition of insulin secretion in INS-1 cells. Cells were incubated and insulin was measured as described in Materials and methods. *Control* denotes incubations performed in the presence of 0.5 mmol/l glucose; *Stimulators* indicates incubations in the presence of 16.7 mmol/l glucose, 5 μmol/l forskolin and 50 μmol/l tolbutamide. Results are mean ± SEM for the number of observations indicated in *parentheses* (3–4 observations per individual experiment)

Conditions	Insulin Release (% of content)	
Control	0.32 ± 0.03	(28)
Stimulators	4.04 ± 0.26	(28)
Stimulators + 1 μmol/l clonidine	1.51 ± 0.30	(8)
Stimulators + 1 μmol/l adrenaline	0.54 ± 0.06	(28)
Stimulators + 1 μmol/l adrenaline + 10 μmol/l rauwolscine	6.22 ± 0.59	(24)
Stimulators + 1 μmol/l adrenaline + 10 μmol/l prazosin	1.46 ± 0.13	(7)
Stimulators + 1 μmol/l adrenaline + 10 μmol/l propranolol	1.27 ± 0.30	(11)
Stimulators + 1 μmol/l adrenaline + 10 μmol/l propranolol + 10 μmol/l rauwolscine	4.38 ± 0.63	(12)**

** Not significant compared to the effect of stimulators alone

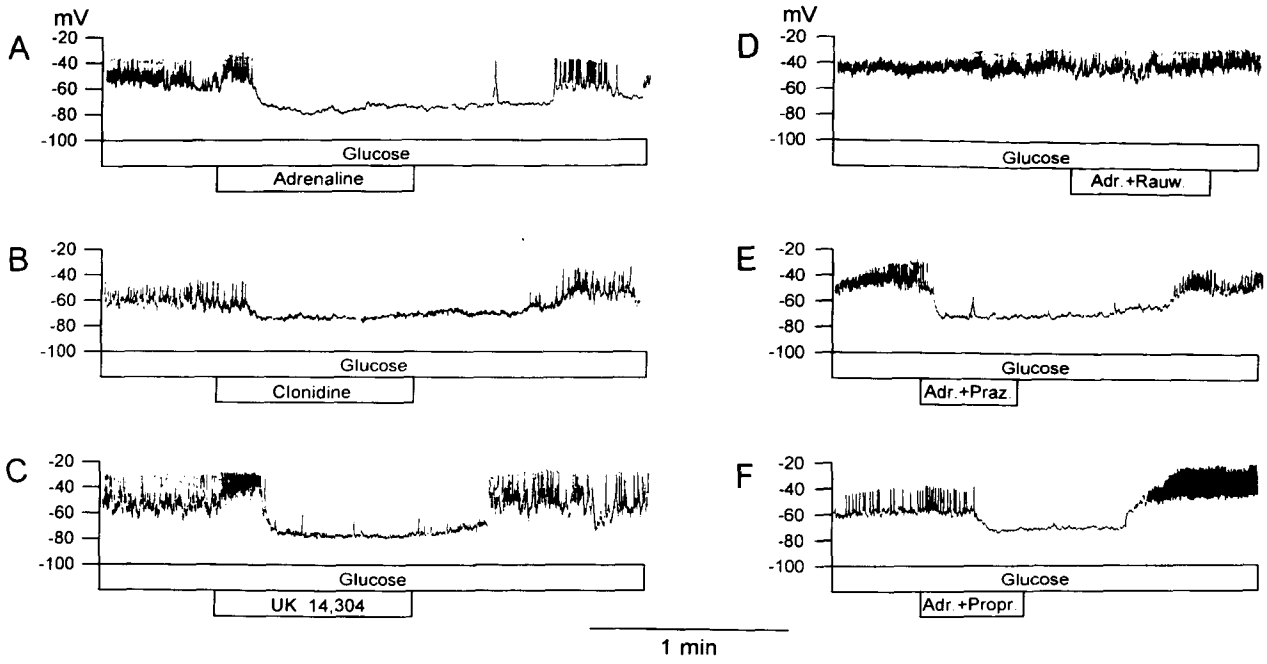

Fig. 2 Effects of adrenoceptor agonists (**A–C**) and antagonists (**D–F**) on glucose-induced depolarization and action potentials in INS-1 cells. Measurements of membrane voltage were performed as described in Materials and methods. The substances were superfused as indicated: **A** 1 μmol/l adrenaline; **B** 1 μmol/l clonidine; **C** 1 μmol/l UK 14,304; **D** 1 μmol/l adrenaline + 10 μmol/l rauwolscine; **E** 1 μmol/l adrenaline + 10 μmol/l prazosin; **F** 1 μmol/l adrenaline + 10 μmol/l propranolol

conductance was 3.9 ± 1.0 nS ($n = 9$) which was reduced to 0.23 ± 0.04 nS by glucose. The addition of adrenaline increased the conductance slightly but significantly to 0.36 ± 0.07 nS ($n = 9$). During these measurements the access conductance was 16 ± 3 nS ($n = 9$). These results suggest that the repolarizing effect of adrenaline is at least in part due to opening of K^+ channels.

Effects of adrenaline and somatostatin on sulphonylurea-sensitive K^+_{ATP} channel activity in INS-1 cells

Since tolbutamide is known to depolarize insulin-secreting cells by its specific inhibitory action on K^+_{ATP} channels present in the plasma membrane, it was important to examine whether adrenaline- and somatostatin-induced hyperpolarization was due to an activation of these channels. Channel analysis of cell-attached patches revealed that tolbutamide-sensitive channels were observed in the plasma membrane of INS-1 cells at 0.5 mmol/l glucose. They have a single-channel conductance of 40–70 pS (mean: 53.8 ± 2.6 pS, $n = 13$) and a reversal potential of 82.4 ± 1.8 mV ($n = 10$, Fig. 4). After addition of tolbutamide, the channels were closed. At the same time, events revealing action potentials appeared (Fig. 5). When adrenaline and somatostatin were added in the presence of tolbutamide, action potentials were abolished but no reopening of these channels could be observed (Fig. 5). Moreover no other channels greater than the basal noise (> 4 pS) were detectable in the presence of adrenaline and somatostatin.

To check whether an adrenaline-evoked effect may have escaped detection, since channels analysed under the patch pipette may not be modulated by adrenaline added to the bath solution, adrenaline was added to the pipette solution only. Under these conditions, we observed similar K^+_{ATP} channel activity to that shown without adrenaline in the pipette and tolbutamide still inhibited channel opening (data not shown).

Thus the repolarizing effect of adrenaline and somatostatin on membrane voltage cannot be explained by an activation of tolbutamide-sensitive K^+_{ATP} channels.

Effects of adrenaline and somatostatin on insulin secretion

Figure 6 demonstrates that adrenaline inhibited insulin secretion induced by glucose (16.7 mmol/l), forskolin (5 μmol/l) and tolbutamide (50 μmol/l) in INS-1 cells. The inhibitory action of adrenaline was half-maximal at 0.05 μmol/l. Adrenaline (1 μmol/l) inhibited secretion by 98%. Clonidine (1 μmol/l) mimicked the effect of the catecholamine (71% inhibition of stimulated secretion, Table 1). Rauwolscine (10 μmol/l) antagonized the catecholamine-evoked effect completely, while prazosin and propranolol, each at 10 μmol/l, did not (Table 1). These results show that adrenaline inhibits

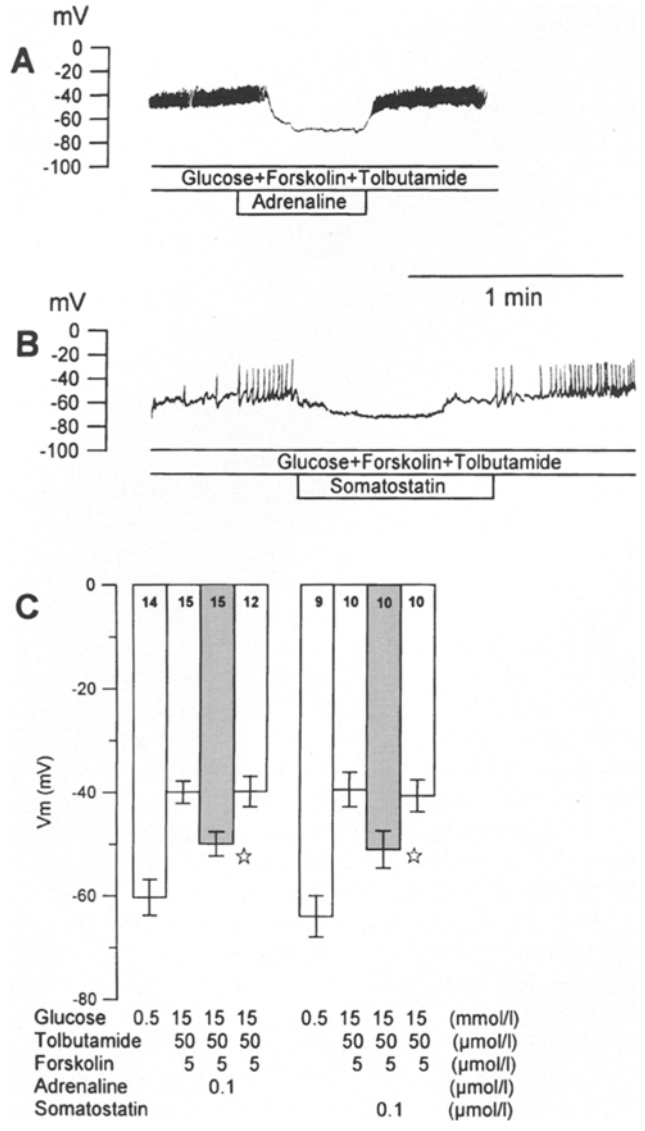

Fig. 3A–C Effects of adrenaline and somatostatin on membrane voltage in the presence of glucose, tolbutamide and forskolin. Measurements of membrane voltage were performed as described in Materials and methods. Cells were superfused with a bath solution containing 15 mmol/l glucose, 50 μmol/l tolbutamide and 5 μmol/l forskolin. **A** Adrenaline (0.1 μmol/l) or **B** somatostatin (0.1 μmol/l) were added as indicated. **C** Mean ± SEM of experiments performed as in **A** and **B** for the number of observations as indicated in each *column*

insulin secretion in INS-1 cells through the activation of α_2-adrenergic receptors. In contrast, somatostatin attenuated insulin secretion by only 30% at 0.1 and 1 μmol/l (Fig. 6).

We also performed experiments with galanin. Galanin did not inhibit stimulated insulin secretion in INS-1 cells nor did it hyperpolarize the INS-1 cell plasma membrane, indicating that either galanin receptors are absent or signal transduction for galanin is interrupted (data not shown).

Discussion

We found that adrenaline and somatostatin repolarize INS-1 cells in the presence of glucose and the sulphonylurea tolbutamide without affecting sulphonylurea-sensitive K^+_{ATP} channel activity (Figs. 3 and 5). These results were surprising since adrenaline abolished secretion in the presence of tolbutamide (Fig. 6) and since it has been found using other insulin-secreting cell lines (RINm5F and HIT-T15) that inhibitory agents such as somatostatin and galanin activate K^+_{ATP} channels [33, 34]. For two reasons it is unlikely that the adrenaline-evoked effects on K^+_{ATP} channels escaped detection in this study. First, when adrenaline was added to the pipette solution only, K^+_{ATP} channel opening was not significantly changed nor was the inhibitory effect of tolbutamide in cell-attached patches. Second, we performed experiments to examine whether adrenaline increases whole-cell conductance in the presence of glucose. Adrenaline augmented whole-cell conductance by only 0.13 nS. As a single K^+_{ATP} channel has a conductance of 0.05 nS, the adrenaline-evoked effect should have to be mediated by only a few K^+_{ATP} channels. In a recent study by Ribalet and Eddlestone [23] performed with RINm5F and HIT-T15 cells, somatostatin activated K^+_{ATP} channels in the absence of glucose, but was unable to reopen K^+_{ATP} channels in the presence of glucose. This finding is in agreement with our results, where glucose and tolbutamide closed channels were not reopened by adrenaline and somatostatin (Fig. 5). The repolarizing effect of inhibitors found in normal β-cells [8, 19] has been attributed to the activation of small K^+ channels distinct from K^+_{ATP} channels [24]. Our results now also suggest that small K^+ channels with a single-channel conductance < 4 pS are activated by adrenaline and are responsible at least in part for the observed repolarization. However, we cannot exclude the possibility that adrenaline has a dual effect on whole-cell conductance, i.e. activation of small K^+ channels and inhibition of Ca^{2+} channels, whereby changes in whole-cell conductance may reflect the sum of both effects. Indeed, adrenaline has been shown to inhibit Ca^{2+} channels resulting in a decrease in cytosolic Ca^{2+} concentration [19] and to inhibit Ca^{2+}-mediated action potentials [27].

Clonidine and UK 14,304 mimicked the adrenaline-evoked effects on membrane voltage and insulin secretion. The α_2-adrenergic antagonist rauwolscine counteracted the adrenaline-evoked effect whereas prazosin and propranolol had only minor effects at very high concentrations. Thus, adrenaline acts through α_2-adrenoceptors. Analysis of the expression pattern of adrenoceptor subtypes in purified rat β-cells suggests that two of three α_2-adrenoceptor subtypes are present in insulin-secreting cells [6]. The receptor subtype expressed in INS-1 cells remains to be determined.

After comparing the effects of adrenaline and somatostatin on membrane voltage, both substances

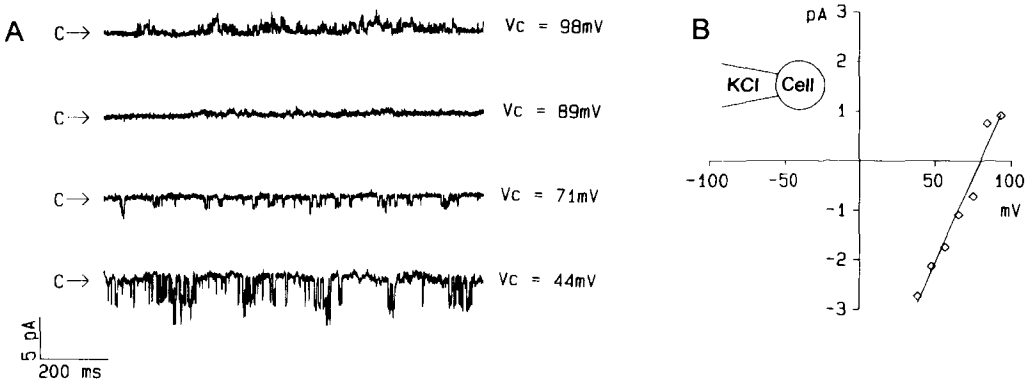

Fig. 4A, B Voltage dependency of channel opening observed in INS-1 cells under control conditions. Measurements were performed in the cell-attached mode as described in Materials and methods. Cells were superfused with bath solution containing 0.5 mmol/l glucose. The patched membrane was clamped with 9 mV steps to ± 50 mV. **B** Current/voltage relationship (*I/V* curve) of channels observed in **A** Analysis was performed as described in Materials and methods

proved to be equipotent. Thus, at 1 μmol/l, the hyperpolarization was only reversed after removal of the agonists. At lower concentrations, both adrenaline and somatostatin induced a transient repolarization similar to that observed for normal β-cells [8, 25]. In INS-1 cells, the duration of the effects and especially the frequency of action potentials varied largely from cell to cell, which is why we felt unwilling to quantify the effects on action potential frequency in more detail. In contrast to the similar potency on membrane voltage, adrenaline was revealed to be much more potent in inhibiting insulin release than somatostatin (Fig. 6). Thus, the effect on membrane voltage alone cannot explain the inhibition of release.

The pertussis toxin sensitivity of adrenaline- and somatostatin-induced membrane repolarization indicates that the effects are mediated via receptors which exert their actions through pertussis-toxin-sensitive G proteins of the $G_{o/i}$ family. Since all other known effects of adrenaline in insulin-secreting cells, the inhibition of adenylyl cyclase, the inhibition of Ca^{2+} channels and the inhibition of insulin release are also mediated via pertussis-toxin-sensitive G proteins [14, 27, 29, 30], toxin treatment cannot discriminate between the different actions. $G_{\alpha i(1-3)}$ and $G_{\alpha o}$ proteins were found in RINm5F, HIT-T15, β-TC and β-cells [16, 27, 28]. G_i proteins were found on insulin-containing granules [16] and G_{i1} was shown to mediate adrenaline-evoked effects on Ca^{2+} channels [27]. It has been suggested previously [30] that adrenaline may exert its inhibition of secretion through a direct coupling of pertussis-toxin-sensitive G proteins to the exocytotic fusion pore. Indeed it has been shown recently that activated G_i and G_o proteins directly inhibit insulin secretion [17]. The results of this study suggest that a distinct set of G proteins and thus different effector systems may be activated by adrenaline and somatostatin. To explain our results, only adrenaline would then stimulate the pathway which directly modulates secretion.

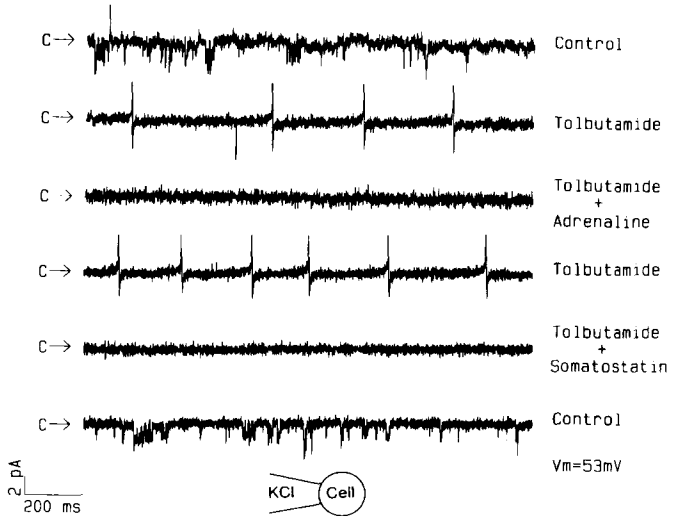

Fig. 5 Effects of tolbutamide, adrenaline and somatostatin on channels in INS-1 cells measured in the cell-attached mode. Cells were superfused with bath solution containing 0.5 mmol/l glucose. Tolbutamide (100 μmol/l), adrenaline (1 μmol/l) and somatostatin (1 μmol/l) were added as indicated. The holding potential of the patch was 53 mV. All traces were from one cell

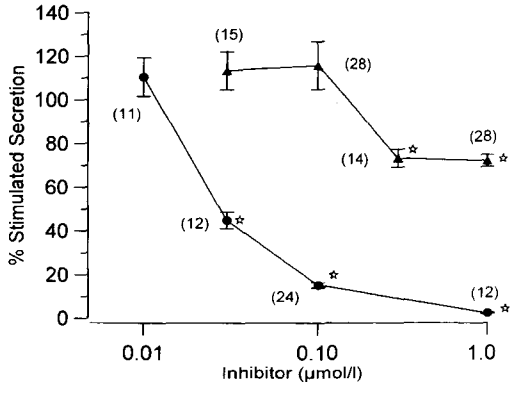

Fig. 6 Effects of adrenaline and somatostatin on glucose-, tolbutamide- and forskolin-induced insulin secretion. Cells were incubated and insulin secretion measured as described in Materials and methods. Curves originate from 3–6 independent experiments for each agonist (●–●, adrenaline, ▲–▲, somatostatin). The number of observations (3–4 for each independent experiment) is given in *parentheses*. Results are expressed as % of release (the increment of secretion induced by glucose, tolbutamide and forskolin was set to 100 %, * significant vs stimulated secretion)

The small inhibitory effect of somatostatin on secretion differs from that found in HIT-T15 cells [32], where 10 nM somatostatin inhibited stimulated release by 85%. It remains to be analysed whether receptor expression and/or the somatostatin-sensitive G protein concentrations are responsible for these differences in distinct cell lines.

The results presented in this study show that repolarization and inhibition of insulin secretion by adrenaline occur in the presence of blocked K^+_{ATP} channels, although these channels are responsible for the membrane voltage in unstimulated insulin-secreting cells. Further analysis of G proteins involved in the different effects of adrenaline and somatostatin may perhaps explain the differences in action of the two inhibitors.

Acknowledgements We thank Dr. R. Greger for helpful discussions. We are gratefully indebted to Ljljiana Mijovic, Kari Zipfel and Charlotte Hupfer for skilled technical assistance. This work was supported by DFG grant Ul 140/1-2. S.U. is a recipient of a DFG fellowship (Ul 140/1-1).

References

1. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP IV, Boyd AE III, González G, Herrera-Sosa H, Nguy K, Bryan J, Nelson DA (1995) Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. Science 268:423–426
2. Amiranoff B, Lorinet AM, Lagny-Pourmir I, Laburthe M (1988) Mechanism of galanin-inhibited insulin release. Eur J Biochem 177:147–152
3. Asfari M, Janjic D, Meda P, Li G, Halban PA, Wollheim CB (1992) Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. Endocrinology 130:167–178
4. Ashcroft SJH, Ashcroft FM (1990) Properties and functions of ATP-sensitive K^+-channels. Cell Signal 2:197–214
5. Bokvist K, Eliasson L, Ämmälä C, Renström E, Rorsman P (1995) Co-localisation of L-type Ca^{2+} channels and insulin-containing secretory granules and its significance for the initiation of exocytosis in mouse pancreatic B-cells. EMBO J 14:50–57
6. Chan SLF, Morgan NG (1995) Molecular analysis of α_2-adrenoceptor gene expression in rat pancreatic A- and B-cells. Diabetologia 38:A130
7. Cook DL, Hales CN (1984) Intracellular ATP directly blocks K^+-channels in pancreatic β-cells. Nature 311:269–271
8. Debuyser A, Drews G, Henquin JC (1991) Adrenaline inhibition of insulin release: role of the repolarization of the B cell membrane. Pflügers Arch 419:131–137
9. Dunne MJ, Bullett MJ, Li GD, Wollheim CB, Petersen OH (1989) Galanin activates nucleotide-dependent K^+ channels in insulin-secreting cells via a pertussis toxin-sensitive G-protein. EMBO J 8:413–420
10. Henquin JC, Meissner HP (1984) Significance of ionic fluxes and changes in membrane potential for stimulus-secretion coupling in pancreatic β-cells. Experientia 40:1043–1052
11. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ (1965) Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375–1384
12. Homaidan FR, Sharp GWG, Nowak LM (1991) Galanin inhibits a dihydropyridine-sensitive Ca^{2+} current in the RINm5F cell line. Proc Natl Acad Sci USA 88:8744–8748
13. Hsu WH, Xiang H, Rajan AS, Kunze DL, Boyd AE III (1991) Somatostatin inhibits insulin secretion by a G-protein-mediated decrease in Ca^{2+} entry through voltage-dependent Ca^{2+} channels in the beta cell. J Biol Chem 266:837–843
14. Katada T, Ui M (1981) Islet-activating protein. A modifier of receptor mediated regulation of rat islet adenylate cyclase. J Biol Chem 256:8310–8317
15. Kerst G, Fischer KG, Normann C, Kramer A, Leipziger J, Greger R (1995) Ca^{2+} influx induced by store release and cytosolic Ca^{2+} chelation in HT_{29} colonic carcinoma cells. Pflügers Arch 430:653–665
16. Konrad RJ, Young RA, Record RD, Smith RM, Butkerait P, Manning D, Jarett L, Wolf BA (1995) The heterotrimeric G-protein G_i is localized to the insulin secretory granules of the β-cells and is involved in insulin exocytosis. J Biol Chem 270:12869–12876
17. Lang J, Nishimoto I, Okamoto T, Regazzi R, Kiraly C, Weller U, Wollheim CB (1995) Direct control of exocytosis by receptor-mediated activation of the heterotrimeric GTPases G_i and G_o or by the expression of their active G_γ subunits. EMBO J 14:3635–3644
18. Morgan NG, Montague W (1985) Studies on the mechanism of inhibition of glucose-stimulated insulin secretion by noradrenaline in rat islets of Langerhans. Biochem J 226:571–576
19. Nilsson T, Arkhammar P, Rorsman P, Berggren PO (1988) Inhibition of glucose-stimulated insulin release by α_2-adrenoceptor activation is paralleled by both a repolarization and a reduction in cytoplasmic free Ca^{2+} concentration. J Biol Chem 263:1855–1860
20. Nilsson T, Arkhammar P, Rorsman P, Berggren PO (1989) Suppression of insulin release by galanin and somatostatin is mediated by a G-protein. J Biol Chem 264:973–980
21. Prentki M, Matschinsky FM (1987) Ca^{2+}, cAMP and phospholipid-derived messengers in coupling mechanisms of insulin secretion. Physiol Rev 67:1185–1249
22. Reaven GM (1995) Pathophysiology of insulin resistance in human disease. Physiol Rev 75:473–486
23. Ribalet B, Eddlestone GT (1995) Characterization of the G protein coupling of a somatostatin receptor to the K^+_{ATP} channel in insulin-secreting mammalian HIT and RIN cell lines. J Physiol (Lond) 485:73–86
24. Rorsman P, Bokvist K, Ämmälä C, Arkhammar P, Berggren PO, Larsson O, Wahlander K (1991) Activation by adrenaline of a low-conductance G protein-dependent K^+ channel in mouse pancreatic B cells. Nature 349:77–79
25. Santana de Sa S, Ferrer R, Rojas E, Atwater I (1983) Effects of adrenaline and noradrenaline on glucose-induced electrical activity of mouse pancreatic β-cell. Q J Exp Physiol 68:247–258
26. Schmid-Antomarchi H, De Weille J, Fosset M, Lazdunski M (1987) The receptor for antidiabetic sulfonylureas controls the activity of the ATP-modulated K^+ channel in insulin-secreting cells. J Biol Chem 262:15840–15844
27. Schmidt A, Hescheler J, Offermanns S, Spicher K, Hinsch KD, Klinz FJ, Codina J, Birnbaumer L, Gausepohl H, Frank R, Schultz G, Rosenthal W (1991) Involvement of pertussis toxin-sensitive G-proteins in the hormonal inhibition of dihydropyridine-sensitive Ca^{2+} currents in an insulin-secreting cell line (RINm5F). J Biol Chem 266:18025–18033
28. Seaquist ER, Robertson Neal A, Shoger KD, Walseth TF, Robertson RP (1992) G-proteins and hormonal inhibition of insulin secretion from HIT-T15 cells and isolated rat islets. Diabetes 41:1390–1399
29. Ullrich S, Wollheim CB (1984) Islet cyclic AMP levels are not lowered during α_2-adrenergic inhibition of insulin release. Studies with epinephrine and forskolin. J Biol Chem 259:4111–4115
30. Ullrich S, Wollheim CB (1988) GTP-dependent inhibition of insulin secretion by epinephrine in permeabilized RINm5F cells. Lack of correlation between insulin secretion and cyclic AMP levels. J Biol Chem 263:8615–8620

31. Ullrich S, Wollheim CB (1989) Galanin inhibits insulin secretion by direct interference with exocytosis. FEBS Lett 247: 401–404
32. Ullrich S, Prentki M, Wollheim CB (1990) Somatostatin inhibition of Ca^{2+}-induced insulin secretion in permeabilized HIT-T15 cells. Biochem J 270:273–276
33. Weille JR De, Schmid-Antomarchi H, Fosset M, Lazdunski M (1988) ATP-sensitive K^+ channels that are blocked by hypoglycemia-inducing sulfonylureas in insulin-secreting cells are activated by galanin, hyperglycemia inducing hormone. Proc Natl Acad Sci USA 85:1312–1316
34. Weille JR De, Schmid-Antomarchi H, Fosset M, Lazdunski M (1989) Regulation of ATP-sensitive K^+ channels in insulinoma cells: activation by somatostatin and protein kinase C and the role of cAMP. Proc Natl Acad Sci USA 86:2971–2975