Quinine inhibits Ca^{2+}-independent K^+ channels whereas tetraethylammonium inhibits Ca^{2+}-activated K^+ channels in insulin-secreting cells

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The effects of quinine and tetraethylammonium (TEA) on single-channel K^+ currents recorded from excised membrane patches of the insulin-secreting cell line RINm5F were investigated. When 100 μM quinine was applied to the external membrane surface K^+ current flow through inward rectifier channels was abolished, while a separate voltage-activated high-conductance K^+ channel was not significantly affected. On the other hand, 2 mM TEA abolished current flow through voltage-activated high-conductance K^+ channels without influencing the inward rectifier K^+ channel. Quinine is therefore not a specific inhibitor of Ca^{2+}-activated K^+ channels, but instead a good blocker of the Ca^{2+}-independent K^+ inward rectifier channel whereas TEA specifically inhibits the high-conductance voltage-activated K^+ channel which is also Ca^{2+}-activated.

RINm5F cell *Patch clamp* *K^+ channel* *Tetraethylammonium* *Quinine*

1. INTRODUCTION

The rhythmic electrical activity evoked by glucose in pancreatic islet cells is closely involved in the regulation of insulin secretion [1,2] and depends amongst other factors on variations in K^+ conductance [3,4]. Following the report that quinine inhibits the Ca^{2+}-induced increase in human red cell K^+ permeability [5] there have been numerous studies showing that quinine reduces K^+ conductance, membrane potential and ^{86}Rb outflux in pancreatic islet cells as well as having glucose-like effects on insulin secretion [4,6–10]. In all these studies it has been assumed that quinine is a specific inhibitor of Ca^{2+}-activated K^+ permeability (conductance). Recent patch-clamp studies on pancreatic islet cells have directly shown the existence of both Ca^{2+}- and voltage-activated K^+ channels with a unit conductance of about 250 pS [11–13] and Ca^{2+}-independent K^+ channels which show inward current rectification with a maximal unit conductance of about 50 pS [14,15]. With the help of patch-clamp single-channel current recording from outside-out membrane patches from the insulin-secreting cell line RINm5F [16,17] we now show that quinine is not a specific inhibitor of Ca^{2+}-activated K^+ channels, but instead completely inhibits the Ca^{2+}-independent inward rectifier K^+ channel at a concentration which has relatively little effect on the large voltage- and Ca^{2+}-activated K^+ channel. Tetraethylammonium (TEA), on the other hand, inhibits the large voltage- and Ca^{2+}-activated K^+ channel at a concentration which has little effect on the inward rectifier K^+ channel.

2. MATERIALS AND METHODS

All experiments were performed on the insulin-secreting cell line RINm5F [16,17]. Cells were

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maintained in RPMI 1640 medium (Flow Labs) containing 11 mM glucose and supplemented with 10% (v/v) heat-inactivated foetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM glutamine. Cells were seeded directly into Falcon type 3001 plastic petri dishes (35 mm diameter) and incubated at 37°C in a humidified atmosphere of 95% O_2/5% CO_2.

All patch-clamp experiments were performed upon excised membrane patches in the outside-out membrane configuration [18]. Current recordings were stored on tape for subsequent replay and analysis. The pipette solution (in contact with the internal surface of the membrane) contained (mM): 140 KCl, 10 NaCl, 1.13 $MgCl_2$, 10 glucose, 10 Hepes and 1 EGTA. No Ca^{2+} was added and the pH was 7.2. The bath solution (in contact with the external surface of the membrane) contained (mM): 140 NaCl, 4.7 KCl, 2 $CaCl_2$, 1.13 $MgCl_2$, 2.5 glucose, 10 Hepes, pH 7.2. Quinine and TEA were added directly to the bath solution when required.

3. RESULTS

Two distinct single-channel K^+ currents have been recorded in excised outside-out membrane patches obtained from RINm5F cells. The first is from an inwardly rectifying channel that has characteristics very similar to those described for a small unit conductance K^+ channel in both cultured neonatal [14] and adult [15] rat pancreatic B-cells, except that after excision of membrane patches the number of active inward rectifier channels slowly declines (fig.1). A similar pattern after patch excision has been recorded for the inwardly rectifying K^+ channel in guinea pig heart cells [19]. The second K^+ channel has a large unit conductance and is voltage- and calcium-activated. The characteristics of this channel are similar to those described in detail for acutely dissociated rat pancreatic islet cells [13] except that a depolarising voltage pulse applied in the virtual absence of internal Ca^{2+} only gives rise to a few brief channel openings (fig.2).

Fig.2 shows that 2 mM TEA added to the solution in contact with the outside of the membrane abolished unitary current steps evoked by voltage activation of the high-conductance K^+ channel. This effect of TEA was completely reversible. In contrast, 2 mM TEA did not influence the level of activity of the inward rectifier channel in the same membrane patch. In the absence of TEA 32 out of 53 voltage jumps applied to this patch resulted in opening of high-conductance (Ca^{2+}-activated) channels, but immediately after the introduction of 2 mM TEA into the bath solution (in contact with the membrane outside) 30 identical voltage jumps failed to elicit any channel openings. In all the 5 outside-out membrane patches in which the effect of TEA was studied, voltage activation of the high-conductance K^+ channel was reversibly abolished by 2 mM TEA whereas current passing through the inward rectifier channels was invariably observed in the same patches.

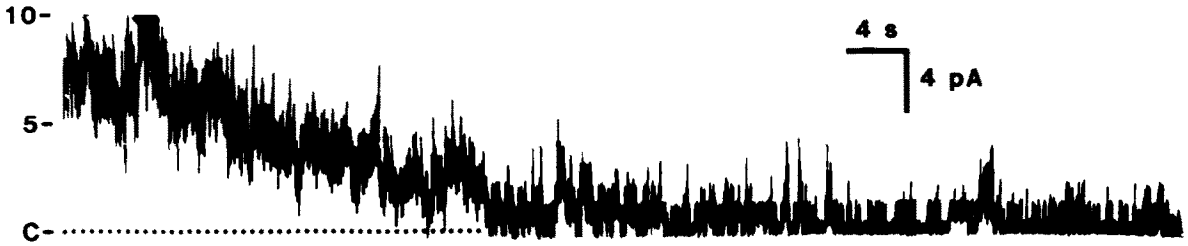

Fig.1. Single-channel current recording from an excised outside-out membrane patch at a membrane potential of 0 mV. Upward deflections represent current flowing from inside of membrane to the outside. The recording starts within 1 min of excision of the membrane patch and shows the existence initially of between 5 and 10 current levels. Gradually the average current flow through the patch decreases and clear single-channel currents can be observed. These unitary currents pass through Ca^{2+}-insensitive inward rectifier K^+-selective channels. At this membrane potential (0 mV) with a very low [Ca^{2+}] in contact with the internal surface of the membrane single-channel currents through high-conductance voltage- and Ca^{2+}-activated K^+ channels cannot be observed.

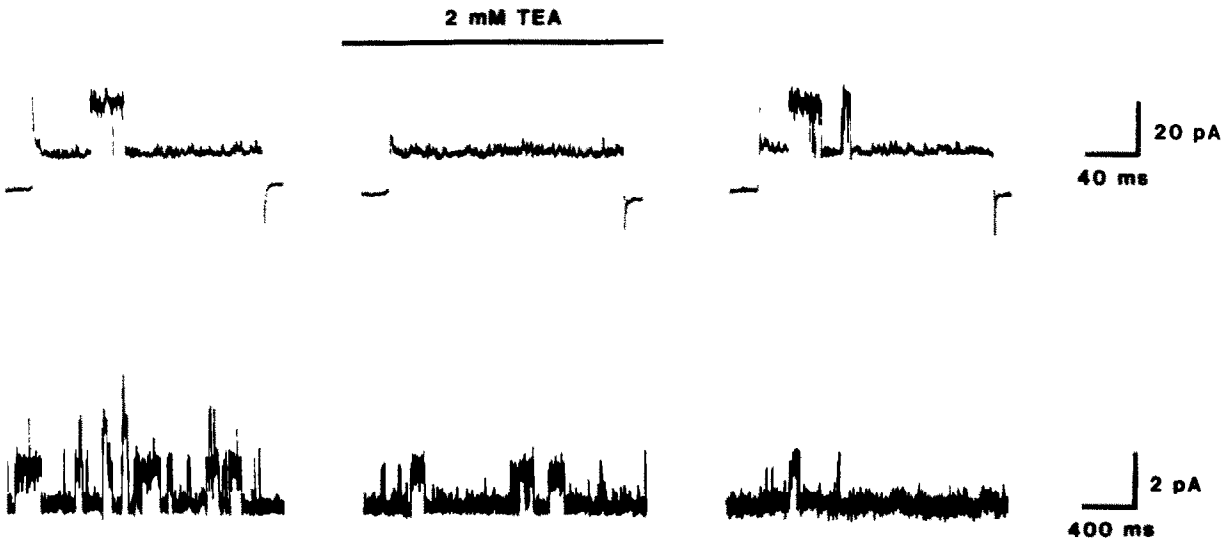

Fig.2. The effect of tetraethylammonium (TEA) on currents through Ca^{2+}- and voltage-activated K^+ channels and inward rectifier K^+ channels. Single-channel current recording from an excised outside-out membrane patch. The upper 3 traces illustrate the currents evoked by carrying out a voltage jump from a membrane potential of 0 to +140 mV. To the left is shown voltage activation of outward K^+ current in the control situation. The middle trace shows that TEA has blocked the voltage-activated single-channel currents. The trace to the right shows that the effect of TEA is rapidly reversible after return to the control solution. The lower 3 traces are recorded from the same membrane patch at a 10-fold higher current gain, but with a slower time base and shows the currents at a membrane potential of 0 mV. The gradual decline of inward rectifier K^+ channel activity is seen but in the presence of TEA (middle trace) clear single-channel currents are still recorded.

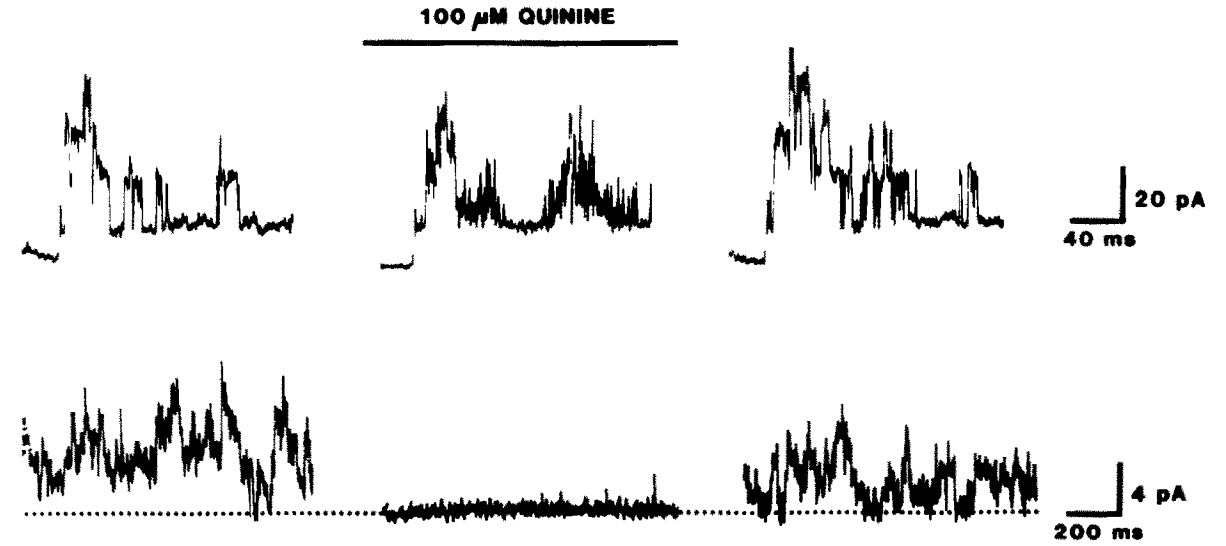

Fig.3. The effect of quinine on currents through Ca^{2+}- and voltage-activated K^+ channels and inward rectifier K^+ channels. Single-channel current recording from an excised outside-out membrane patch. The upper 3 traces illustrate voltage-activation (voltage jump from 0 to +80 mV membrane potential) of the Ca^{2+}-activated high-conductance K^+ channels in the absence and presence of 100 μM quinine and after return to the quinine-free solution. The lower 3 traces from the same patch demonstrate that 100 μM quinine abolishes unitary currents through the inward rectifier K^+ channels. This effect is reversible.

Fig.3 shows that 100 μM quinine abolished the single-channel currents associated with the inward rectifier channels. This effect was reversible. In the same patches this concentration of quinine did not abolish voltage activation of the high conductance K^+ channel, but channel openings were much briefer and could often not be fully resolved when quinine was present. Nevertheless, considerable current flowed through the high conductance K^+ channels during complete inhibition of the inward rectifier channels by quinine. In the experiment shown in fig.3 all the 90 voltage jumps applied to this patch (both before, during and after the application of quinine) activated high-conductance K^+ channels. In the 3 outside-out membrane patches in which the effect of quinine on both types of K^+ channels was studied, 100 μM quinine abolished all current through the inward rectifier channels whereas voltage activation of the high-conductance K^+ channels could be observed in the same patches. In a further 7 outside-out patches quinine (100 μM) abolished activity in the inward rectifier K^+ channels in each case. In this series of experiments voltage activation of high-conductance K^+ channels was not attempted.

4. DISCUSSION

Our results demonstrate directly that quinine in a concentration as high or higher than that used in previous studies on insulin-secreting cells [4,6–10] is not, as has been generally assumed, a specific inhibitor of Ca^{2+}-activated K^+ channels. It has been reported that quinine (100 μM) reduces activity in the ATP-sensitive inward rectifier K^+ channel [14] and the present results now show that it is a relatively specific inhibitor of this Ca^{2+}-independent K^+ channel. TEA inhibits a variety of K^+ channels in other tissues [20], but our results show that when it is used in a low concentration (2 mM) from the outside of the membrane it is a relatively specific inhibitor of the high conductance voltage- and Ca^{2+}-activated K^+ channel.

In the resting intact cell where $[Ca^{2+}]_i$ is about 100 nM [21] there can be virtually no current flowing through Ca^{2+}-activated K^+ channels as the open-state probability at this level of $[Ca^{2+}]_i$ is 0 at normal negative membrane potentials [13]. Activity of the inward rectifier K^+ channels is, however, dependent neither on the membrane potential nor on the magnitude of $[Ca^{2+}]_i$ [14] and this channel dominates the resting cell K^+ permeability [15]. In the light of the results shown in fig.3 it is now clear that the ability of quinine to depolarize the membrane and induce electrical activity, reduce ^{86}Rb efflux and evoke insulin secretion [4,6–10] is due to inhibition of the ATP-sensitive inward rectifier K^+ channels and not as previously assumed to inhibition of Ca^{2+}-activated K^+ channels. These results [3–9] now appear consistent with the recent finding that glucose induces closure of the inward rectifier K^+ channels in intact rat pancreatic B-cells [15]. This does not necessarily mean that the Ca^{2+}-activated K^+ channels are of no importance for the function of the insulin-secreting cells. Whereas the previous evidence based on the quinine studies [4,6–10] suggested that glucose inhibits Ca^{2+}-activated K^+ permeability, it has been shown that glucose activates a Ca^{2+}-dependent K^+ permeability [22]. Since the Ca^{2+}-activated K^+ channels are not operational in the resting cell, but are activated by higher levels of $[Ca^{2+}]_i$ [13] stimulation of insulin secretion by glucose which raises $[Ca^{2+}]_i$ [23,24] is likely to activate the TEA-sensitive channels. With the availability of relatively specific inhibitors for the two most important K^+ channels in the insulin-secreting cells it should now prove possible to elucidate more precisely their relative contributions during various types of stimulation.

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