Differential effects of the K^+ channel blockers apamin and quinine on glucose-induced electrical activity in pancreatic β-cells from a strain of ob/ob (obese) mice

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The effects of apamin and quinine on glucose-induced electrical activity in pancreatic islets from ob/ob mice (Norwich colony) were compared. Apamin (40-400 nM) increased the duration of the bursts of electrical activity, whereas quinine (50-100 μM) affected only slightly the steady-state electrical response to glucose. This sensitivity to apamin and poor response to quinine contrast with the resistance to apamin and sensitivity to quinine previously reported for pancreatic islets from albino mice. The results give further support to the idea that pancreatic β-cells from ob/ob mice have a modified Ca^{2+}-activated K^+ permeability.

Apamin *Quinine* *Pancreatic β-cell* *ob/ob mouse* *Electrical activity* *Ca^{2+}-activated K^+ permeability*

1. INTRODUCTION

A Ca^{2+}-activated K^+ permeability (P_K-Ca) has been proposed to play a key role in the regulation of the burst pattern of electrical activity in pancreatic islets stimulated by glucose [1–3]. In islets of Langerhans isolated from both Wistar rats and albino mice, P_K-Ca has been shown to be blocked by quinine [2] and resistant to apamin [4], a bee venom neurotoxin which has been reported to block P_K-Ca in other cell types ([5–8], review [16]). Recently, pancreatic islets from ob/ob mice (Norwich colony) were shown to exhibit a modified pattern of electrical response to glucose and to be poorly sensitive to quinine [9]. In an attempt to further characterize the properties of P_K-Ca in the ob/ob β-cell, the effects of apamin and quinine on glucose-induced electrical activity were compared in islets from the same strain of ob/ob mice.

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2. MATERIALS AND METHODS

Homozygous ob/ob (obese) brown mice originally obtained from the Institute of Animal Genetics at the University of Edinburgh, and since then maintained as an out-bred colony in the animal house of the School of Biological Sciences, University of East Anglia (Norwich, England) [9] were used in this study. The ob/ob islets had an average diameter 4–5-times the regular size of islets from albino mice (from now on referred to as normal mice).

The electrical activity was recorded with high-resistance microelectrodes as reported [10]. The chamber for the microdissected islets of Langerhans was perfused with a modified Krebs solution containing 120 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl, 2.56 mM $CaCl_2$ and 1.13 mM $MgCl_2$. The solution was continuously gassed with a mixture of O_2 (95%) and CO_2 (5%) at 37°C, to keep the pH at 7.4.

Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and quinine hydrochloride were from Sigma (London). Purified apamin was a generous gift from Drs M. Lazdunski and M. Claret.

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3. RESULTS

In islets of Langerhans from ob/ob mice, glucose (11 mM) induced electrical activity in the form of bursts consisting of an active phase at a depolarized level of −35 mV, and a silent phase at −55 mV (fig.1, upper record). The effects of apamin (200 nM) on this glucose-induced electrical activity in the ob/ob β-cell are shown in fig.1 (lower record). Apamin increased the duration of the burst active phase and, in addition, the pattern of spike activity along the active phase of a burst was changed to an irregular pattern in which the spikes were separated by silent periods of variable duration.

The effects of increasing apamin concentration on glucose-induced electrical activity are illustrated in fig.2. As in other ob/ob β-cells, bursts of variable duration were recorded (see [9]). Addition of 4 nM apamin slightly increased the spike amplitude, whilst exposing the same islet to 40 nM apamin clearly increased the time spent in the active phase. Although it is not immediately apparent from fig.2, 40 nM apamin also increased spike amplitude and generated an irregular spike pattern similar to that observed in fig.1. It may also be seen in fig.2 (end of middle record) that the burst pattern of electrical activity recovered in less than 1 min after the removal of apamin. However, the duration of the active phase then slowly increased until electrical activity became continuous (fig.2, beginning of lower record). Finally, the islet was exposed to 400 nM apamin with the purpose of recording the effects of CCCP in the presence of the toxin. It may be seen that the β-cell membrane remained depolarized throughout exposure to 400 nM apamin, and that the amplitude of the spikes was further increased. Addition of CCCP (5 μM) gradually suppressed spike activity but did not hyperpolarize the β-cell membrane.

The effects of quinine on glucose-induced electrical activity in ob/ob mouse islets were tested at low drug concentrations to minimize the possible effects of the drug on islet cell metabolism [11] and

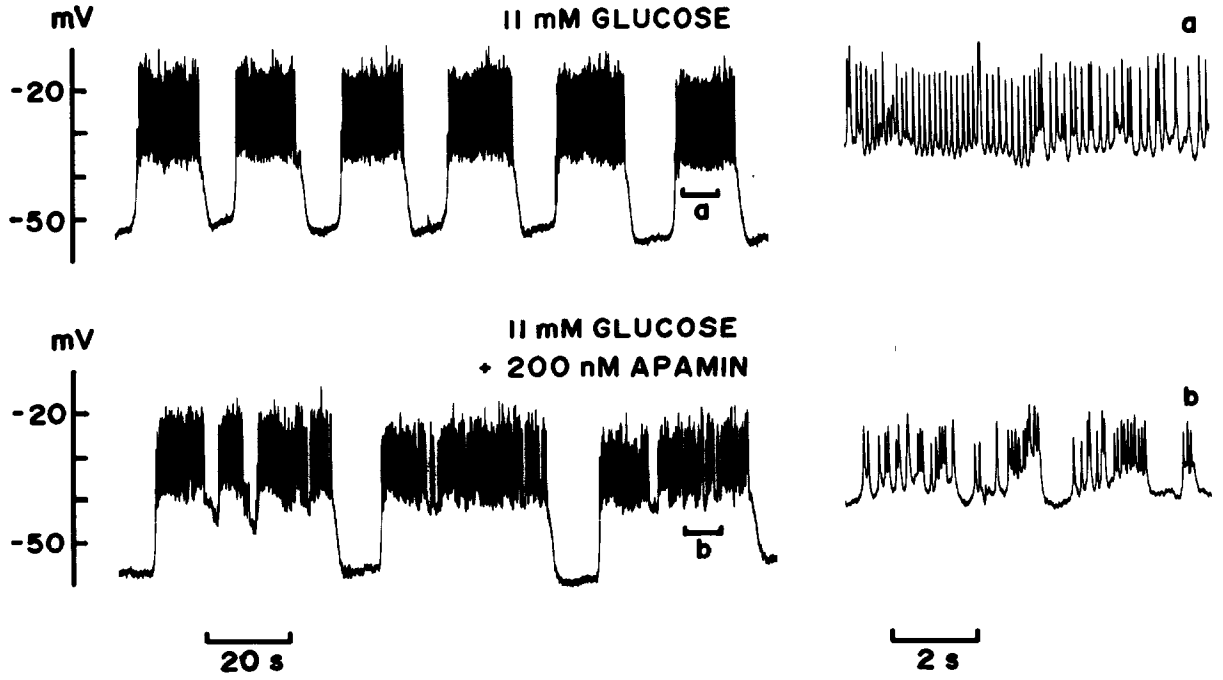

Fig.1. Effects of apamin on the burst and spike characteristics of an ob/ob β-cell. Upper left record: burst activity of an ob/ob β-cell in the presence of 11 mM glucose in Krebs medium. Lower left record: a record of electrical activity from the same cell 5 min after adding 200 nM apamin to the medium. Expanded sample records (10 ×) illustrating details of the spikes are shown to the right of each record.

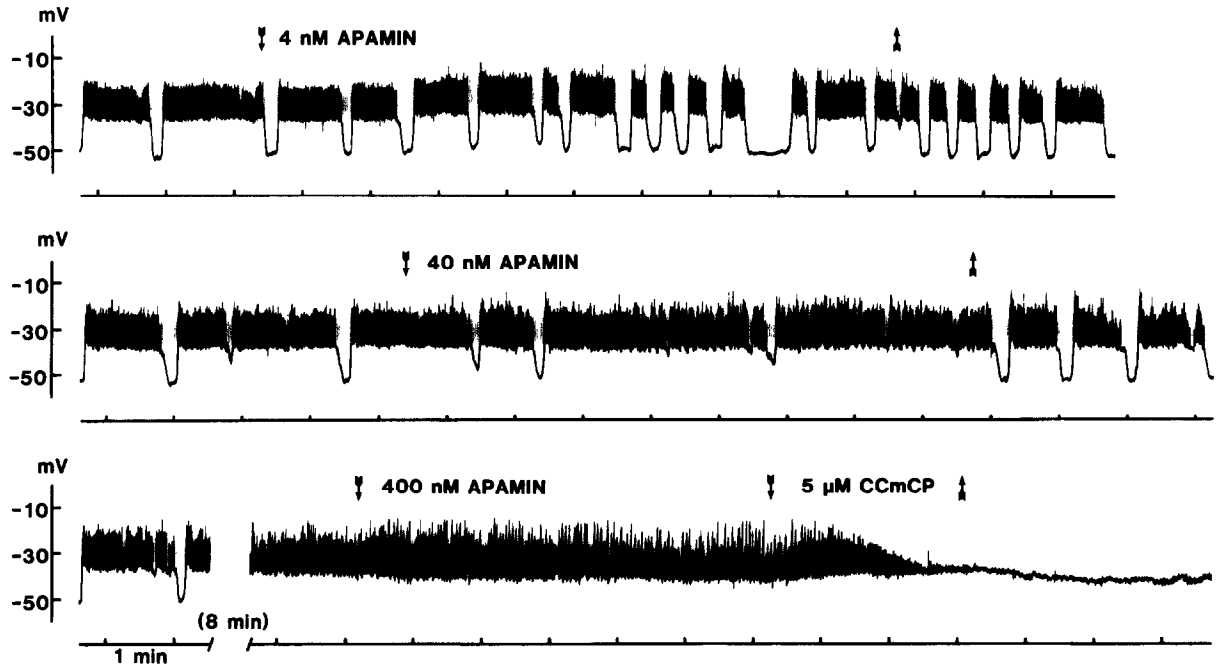

Fig.2. Effects of increasing concentrations of apamin on glucose-induced electrical activity in an ob/ob β-cell. Three consecutive records from the same cell showing the effect of exposure of the islet to 4, 40 and 400 nM apamin as indicated by the arrows in the presence of 11 mM glucose. CCCP (5 μM) was added during continued exposure to 400 nM apamin.

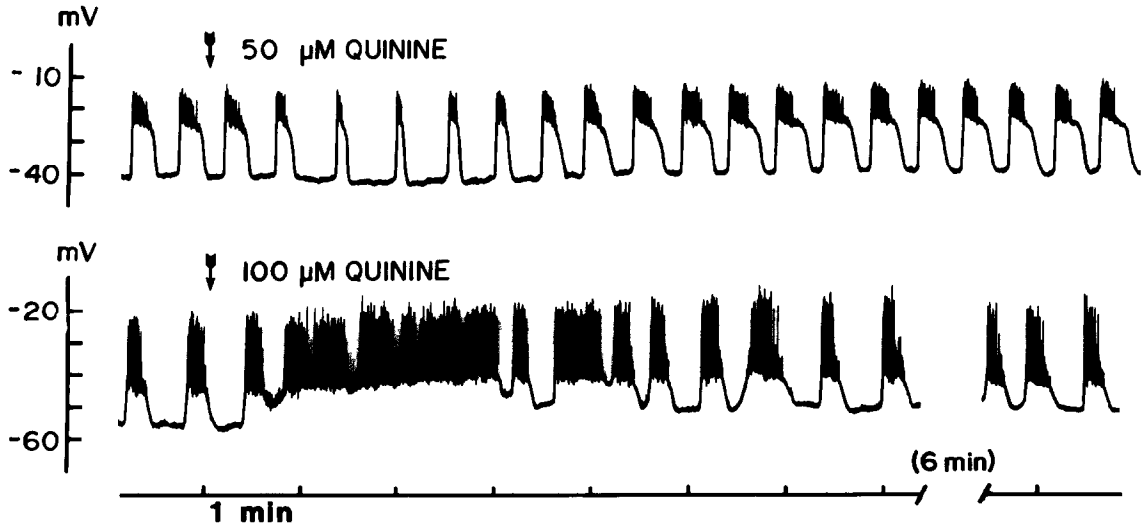

Fig.3. Effects of quinine on glucose-induced electrical activity in the ob/ob β-cell. Upper record: effect of 50 μM quinine in the presence of 11 mM glucose. Lower record: effect of 100 μM quinine on a different islet also stimulated by 11 mM glucose. Notice the longer application time of 100 μM quinine.

ionic permeabilities other than P_K-Ca, as reported for its optical isomer quinidine [12]. Fig.3 (upper record) shows that after a transient reduction of glucose-induced electrical activity characterized by short bursts and long silent phases, 50 μM quinine slightly increased the fraction of time in the active phase and slightly reduced the membrane potential at the silent phase. In the presence of 100 μM quinine, the ob/ob β-cell membrane became transiently depolarized to a level close to the active phase potential (fig.3, lower record). This effect, which lasted about 2 min, was followed by the resumption of the burst pattern of electrical activity characterized by a slightly depolarized silent phase and an active phase duration similar to that measured in the control. In contrast, in islets from normal mice quinine (50–100 μM) blocked the burst silent phase and induced continuous electrical activity (see [2]).

4. DISCUSSION

The present data show that apamin, but not quinine, has a marked effect on the burst pattern of electrical activity induced by glucose in pancreatic islets from ob/ob mice (Norwich colony). In contrast, both $^{86}Rb^+$ efflux from pre-loaded rat islets and electrical activity in pancreatic islets from normal mice are sensitive to quinine, but resistant to apamin [4].

The results suggest that apamin, which specifically blocks P_K-Ca in several cell types [5–8], may also block this K^+-permeability in ob/ob islets. Indeed, exposure to apamin depolarized the ob/ob β-cell membrane as is the case for quinine in islets from normal mouse [2] and, furthermore, the toxin impaired the hyperpolarizing effect of CCCP. This uncoupler of oxidative phosphorylation has been reported to hyperpolarize the β-cell membrane through activation of P_K-Ca, leading to cessation of spike activity, both in islets from albino mice [14] and from ob/ob mice [9]. In addition, apamin affected the spike characteristics. Thus, the toxin changed the continuous spike activity along the active phase of the burst into an irregular pattern and, in some experiments, it also increased spike amplitude. These effects may result from the blockade of a Ca^{2+}-activated K^+ channel in the ob/ob β-cell. In the normal β-cell, P_K-Ca has been claimed to participate in spike repolarization [13].

Although in different cell types the dissociation constant for the apamin-channel complex is in the range from 15 to 60 pM [7,8,16], relatively high apamin concentrations (40–400 nM) were required to induce a clear effect in ob/ob β-cells. Therefore, it is possible that either the number of apamin binding sites or the affinity of the apamin channel receptors is low in ob/ob β-cells. It is also possible that 2 distinct types of Ca^{2+}-activated K^+ channels might coexist in the ob/ob β-cell, as reported for rat muscle cells [15]. It seems therefore that the ob/ob β-cell, unlike the normal β-cell, is equipped with more apamin-sensitive channels than quinine-sensitive channels. Thus, the ob/ob β-cell would resemble cells exhibiting an apamin-blockable Ca^{2+}-activated K^+ channel, i.e. hepatocytes, neuroblastoma, smooth muscle and skeletal muscle cells [5–8].

The reason why the ob/ob β-cell electrical response to apamin is variable from islet to islet is not clear. Although a variable density of apamin channel receptors could possibly account for such variability, it is also possible that the accessibility of the high-M_r toxin molecule (M_r = 2032) to the electrically monitored islet cell might be related to other variable factors such as the islet size.

Finally, the finding that ob/ob islets contain apamin-sensitive Ca^{2+}-activated K^+ channels gives further support to the hypothesis that the P_K-Ca of ob/ob β-cells differs from that of β-cells from normal mouse [9]. This modified K^+ permeability may play a role on the diabetes-like syndrome in ob/ob mice [17].

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