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Inhibition of electrical activity in mouse pancreatic β-cells by the ATP/ADP translocase inhibitor, bongkrekic acid

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Bongkrekic acid causes fatal food poisoning which is associated with hyperglycaemia. Here we demonstrate that bongkrekic acid, a potent inhibitor of the mitochondrial ATP/ADP translocase, inhibits glucose-induced electrical activity in the pancreatic β-cell through the stimulation of ATP-sensitive potassium channel (K-ATP-channel) activity. By comparison of its effects with those of oligomycin, we suggest that bongkrekic acid acts by the inhibition of glucose metabolism and may induce hyperglycaemia by impairing β-cell function.

Bongkrekic acid; Metabolism; ATP-sensitive potassium channel; Pancreatic β-cell

1. INTRODUCTION

Bongkrekic acid is a toxic antibiotic produced through a secondary infection by the bacteria *Pseudomonas cocovenens* of partially defatted coconut [1]. Bongkrekic acid is a potent inhibitor of oxidative metabolism in both heart [2] and liver mitochondria [3], an effect which is attributed to specific inhibition of the mitochondrial ADP/ATP translocase [4].

Glucose stimulates insulin secretion from pancreatic β-cells. This is associated with electrical activity which plays a key role in this process. Glucose metabolism stimulates electrical activity, and thus insulin secretion, by inhibiting K-ATP channels [5,6]. This latter process is thought to occur by an increase in the cytosolic ATP/ADP ratio [6,7]. Agents which inhibit glucose metabolism reverse the effects of glucose on both insulin secretion and the K-ATP channel. This raises the possibility that the hyperglycaemia in bongkrekic acid poisoning results from an impairment of insulin secretion as a consequence of an inhibition of glucose metabolism. We have therefore studied the action of bongkrekic acid on the electrophysiology of pancreatic β-cells in an attempt to understand the hyperglycaemia associated with 'bongkrek' poisoning.

2. MATERIALS AND METHODS

For intracellular recording, pancreatic islets were microdissected from NMRI mice and were perifused with the following solution (in mM): 120 NaCl, 25 $NaHCO_3$, 5 KCl, 2.56 $CaCl_2$ and 1.1 $MgCl_2$. The solution was continuously gassed with a mixture of 95% O_2/5% CO_2 at 37°C (pH 7.4). The techniques for recording β-cell membrane potential using intracellular microelectrodes have been described elsewhere [8,10]. The input resistance was determined by injecting alternate depolarizing and hyperpolarizing current steps during the phases of electrical silence and measuring the passive voltage response [8,10]. Patch-clamp studies were made on primary cultured β-cells prepared as previously described [11,12]. Single cells were studied at both 20°C and 30°C in a solution containing (in mM): 138 NaCl, 5.6 KCl, 2.6 $CaCl_2$, 1.1 $MgCl_2$, 10 NaOH-HEPES (pH 7.4). In the cell-attached experiments the pipette contained (in mM): 140 KCl, 2.6 $CaCl_2$, 1.2 $MgCl_2$, 10 KOH-HEPES (pH 7.4). For the whole cell experiments the pipette contained (in mM): 107 KCl, 10 NaCl, 2 $MgCl_2$, 30 KOH, 10 EGTA, 10 KOH-HEPES, 1 $CaCl_2$ (pH 7.2). 0.3 mM Na_2ATP was included in the pipette solution to reduce rundown of K-ATP-channels [12]. Currents were recorded using a List EPC-7 amplifier (List, Darmstadt, Germany), filtered at 0.5 kHz (8-pole Bessel; Frequency Devices) and were digitised at 1 kHz (PCLAMP, Axon Instruments, Burlingame, USA). They were analysed using PCLAMP software. Both oligomycin and tolbutamide were from Sigma. Diazoxide was a gift from Glaxo Laboratories. Bongkrekic acid was a gift from Dr. Le Quoc, Laboratoire de Biochemie, UA CNRS, Bescancon, Cedex-France.

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3. RESULTS

Fig. 1A illustrates the membrane potential response of a β-cell in an isolated islet to 11 mM glucose. In the presence of the sugar the β-cell produces rhythmic bursts of action potentials separated by hyperpolarized, electrically silent, periods at -52 ± 3 mV ($n = 4$). Within 1 min of removing glucose the membrane potential slowly hyperpolarized to -65 ± 3 mV ($n = 4$), the input resistance decreased to 57 ± 8% ($n = 4$) of that measured in 11 mM glucose, indicating an increase in a hyperpolarizing conductance. Adding back glucose

produced opposite changes in membrane potential and input resistance, and gave rise to the characteristic biphasic pattern of β-cell electrical activity. This activity consists of an initial phase of continuous action potential firing which then transforms into bursting activity.

Fig. 1B shows that when 128 μM bongkrekic acid was added to an islet bursting in 11 mM glucose, it caused a rapid hyperpolarization to −65 mV ± 3 (n = 4) and inhibited electrical activity. The input resistance decreased to 95 ± 6% (n = 4) of that measured in 0 mM glucose. These effects are similar to those obtained on removing glucose (Fig. 1A). The effects of bongkrekic acid were reversible. These effects of bongkrekic acid suggest that it inhibits β-cell glucose utilisation.

In islets, oligomycin, a potent blocker of respiration, inhibits glucose oxidation and lowers intracellular ATP [7]. Oligomycin (2 μg/ml) hyperpolarized the membrane potential to −67 ± 5 mV (n = 4) and inhibited glucose-induced electrical activity. These effects are similar to those of bongkrekic acid (Fig. 1B,C). The input-resistance decreased to 92 ± 10% (n = 3) of that measured in 0 mM glucose. These effects are similar to those reported when glucose is removed or its metabolism impaired by other mitochondrial [9] or glycolytic inhibitors [8,10]. The effects of oligomycin, unlike bongkrekic acid, were irreversible.

The effects of bongkrekic acid on K-ATP-channels were studied in cell-attached membrane patches [12]. Fig. 2A illustrates K-ATP-channel activity from a cell-attached patch at 30°C. In the absence of glucose, channel openings were inward with an amplitude of −4 pA. The single-channel current/voltage relationship in 0 mM glucose showed inward rectification with a slope con-

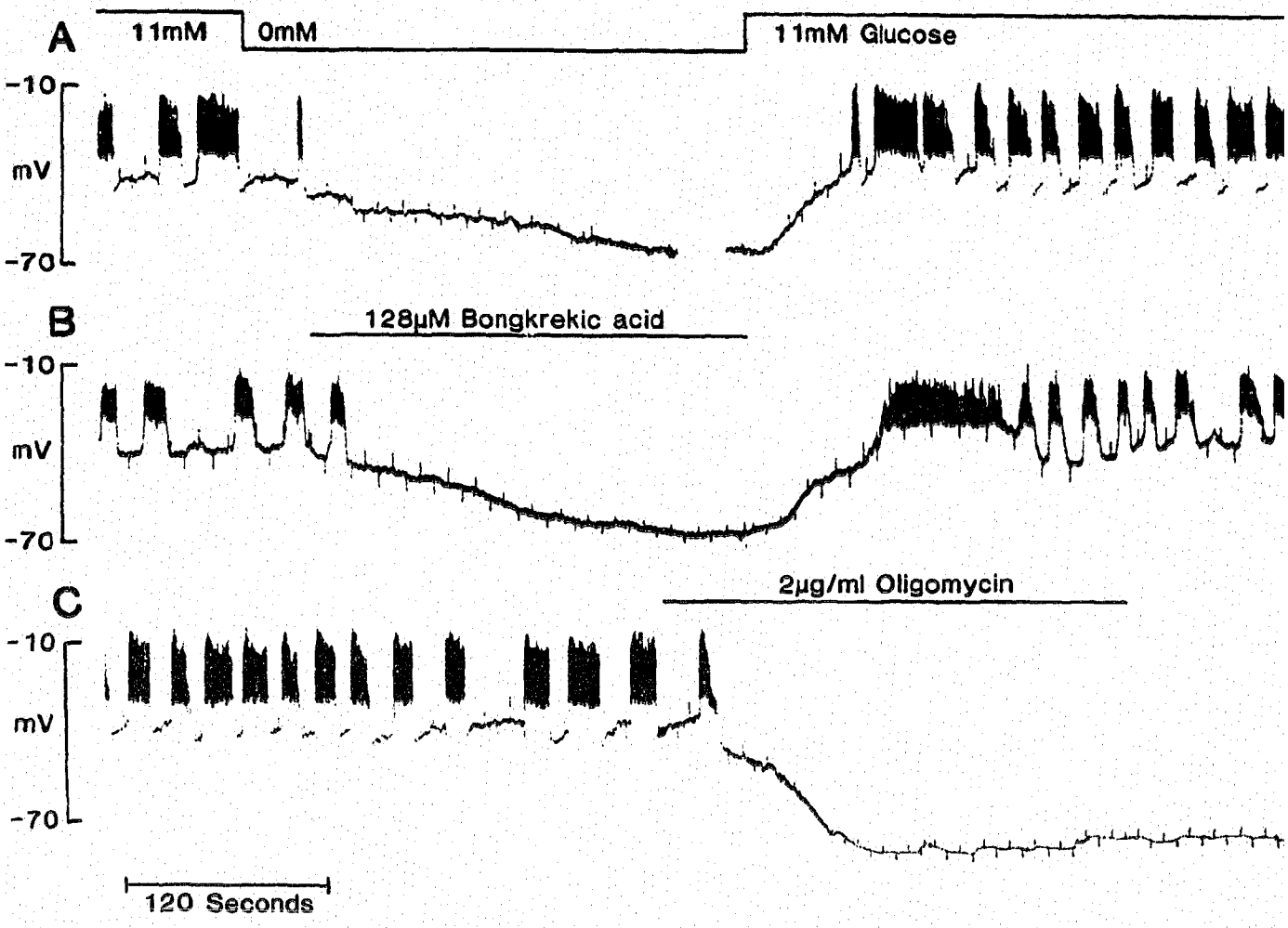

Fig. 1. Membrane potential recorded from an isolated islet in response to 11 mM glucose (A). Effect of bongkrekic acid (B) or oligomycin (C on the electrical activity induced by 11 mM glucose. Small alternate voltage deflections are the membrane response to current injection (see sectio 2). 2 min of record is omitted from (A) to aid comparison with (B). In (C), the cell remained hyperpolarized until the recording was lost. (A) an (B) same cell.

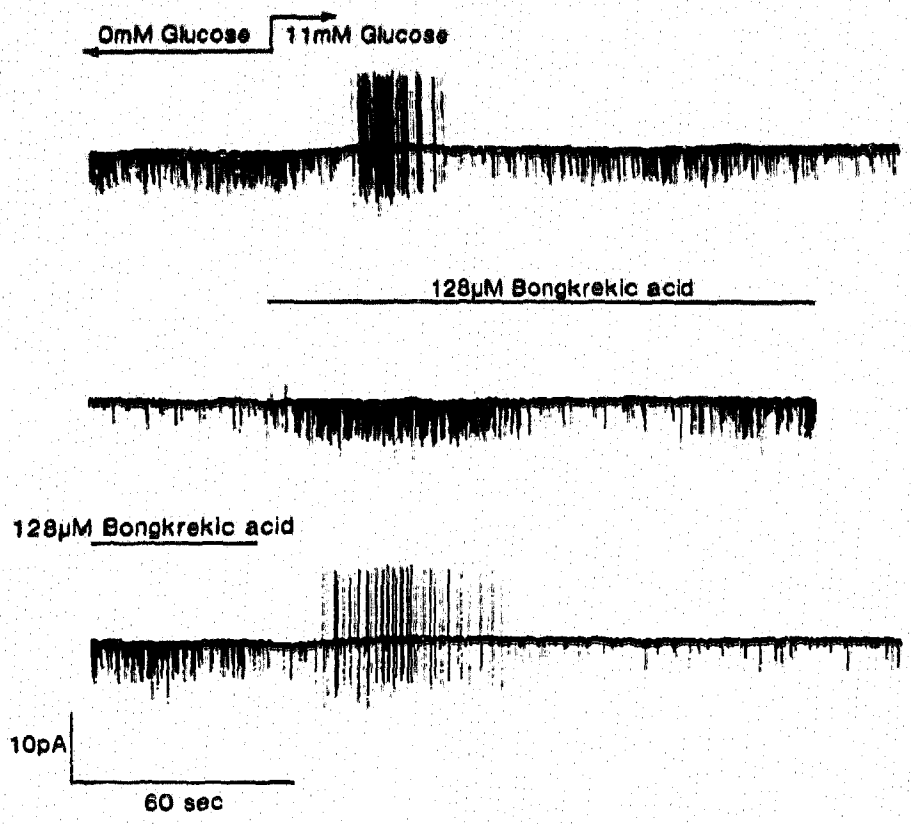

Fig. 2. K-ATP-channel activity recorded at a pipette potential of 0 mV from a cell-attached patch at 30°C. Small downward deflections are channel openings, large bipolar deflections are action currents. Response to 11 mM glucose and the subsequent addition of bongkrekic acid (bars). Records are continuous.

ductance of 57 ± 0.6 pS (n = 3) and a reversal potential of −74 ± 5.2 mV (n = 5), properties consistent with those of the glucose-sensitive channel (K-ATP-channel) that are well described in β-cells [5,11–13]. Addition of 11 mM glucose produced a gradual reduction in both channel activity and the single-channel current amplitude. The reversal potential depolarized by 10–20 mV to −45 ± 17 (n = 3); this voltage change is similar to that produced by glucose with the microelectrode recordings [12,13]. Biphasic action-currents, arising from action potentials in the unclamped region of the cell membrane, were also observed (Fig. 2). These changes occurred within 1 min of adding glucose, a period that is substantially faster than the 3–4 min previously reported for similar effects at room temperature [11]. 128 μM bongkrekic acid produced a rapid increase in both K-ATP-channel activity and single-channel current amplitude. The latter is due to hyperpolarization of the β-cell membrane potential caused by the activation of the K-ATP-channel. The effects of bongkrekic acid were fully reversible. Similar results were observed in 3 out of 4 cells with 128 μM bongkrekic acid and 2 out of 2 cells with 64 μM bongkrekic acid. Oligomycin (2 μg/ml) also relieved the inhibition of K-ATP-channel activity induced by 11 mM glucose (n = 4, not shown), an effect indistinguishable from that of bongkrekic acid.

To determine that bongkrekic acid was acting via the inhibition of metabolism and not by direct activation of K-ATP-channels we recorded whole-cell currents. In this method intracellular constituents of metabolism are washed out of the cell [12]. Whole-cell K-ATP-currents were elicited by a pulse protocol similar to that used by Trube et al. [12]. We attributed the current to that flowing through K-ATP-channels for the following reasons. (i) A rapid increase in current amplitude on achieving the whole-cell configuration. Dialysis of intracellular ATP causing a relief of channel inhibition [12]. (ii) Rapid and reversible inhibition of this current by 100 μM tolbutamide, a specific and potent blocker of K-ATP-channels in β-cells (Fig. 3B, n = 4) [12]. (iii) Rapid and reversible augmentation of this current by 100 μM diazoxide, a specific opener of K-ATP-channels in β-cells (Fig. 3C, n = 3) [12]. Bongkrekic acid (128 μM) was without effect in 6 cells tested (Fig. 3A). This suggests that the drug does not act as a direct activator of the K-ATP-channel. We cannot rule out, however, the

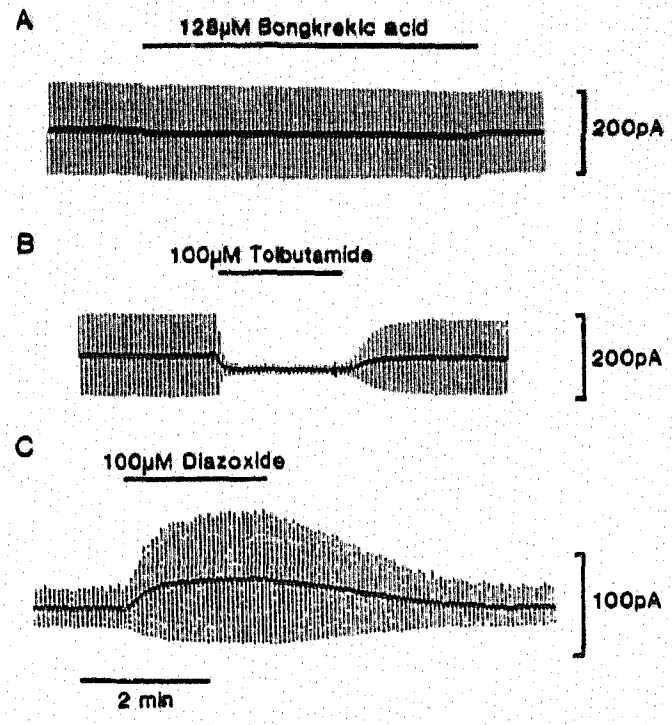

Fig. 3. Whole-cell ATP-sensitive potassium currents elicited by alternate ± 10 mV voltage steps (200 ms duration, 0.5 Hz) from a holding potential of −70 mV. (A) Effect of bongkrekic acid. (B) Inhibition of K-ATP currents by 100 μM tolbutamide. (C) Activation of K-ATP currents by 100 μM diazoxide. (A) and (B) same cell.

possibility that a cytosolic component, washed out in the whole-cell configuration, is required for a direct action of bongkrekic acid on the channel.

4. DISCUSSION

We have shown that bongkrekic acid is a potent and reversible inhibitor of glucose-induced electrical activity in the pancreatic β-cell. This effect is mediated by activation of K-ATP-channels. The effect of bongkrekic acid cannot be attributed to a direct action as a channel opener like diazoxide as it has no effect on whole-cell recordings. The similar effects of oligomycin support the idea that bongkrekic acid is acting via the inhibition of glucose metabolism. This is consistent with the ability of bongkrekic acid to inhibit the ATP/ADP translocase which can be expected to lower the cytosolic ATP/ADP ratio and thereby activate K-ATP-channels.

The profound inhibition of β-cell electrical activity by bongkrekic acid may be expected to result in inhibition of glucose-induced insulin secretion [6]. Whether the hyperglycaemia associated with 'bongkrek' poisoning is due to hypoinsulinaemia remains to be determined as insulin levels in 'bongkrek' poisoning are not known.

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