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Curcumin induces electrical activity in rat pancreatic β-cells by activating the volume-regulated anion channel

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ABSTRACT

Curcumin, the principal active component of turmeric, is reported to exert a number of therapeutic actions, including a hypoglycaemic/antidiabetic action. The underlying mechanisms to this action are essentially unknown. We have investigated the hypothesis that a direct stimulatory action on the pancreatic β-cell could contribute towards the hypoglycaemic activity of this compound. Electrical and ion channel activity were recorded in rat β-cells using the patch–clamp technique. β-Cell volume was measured using a video-imaging technique. Insulin release was measured from intact islets by radioimmunoassay. Curcumin (2–10 μM) activated the volume-regulated anion channel in β-cells. Single channel studies indicated that activation was the result of increased channel open probability. This effect was accompanied by depolarisation of the cell membrane potential, the generation of electrical activity and enhanced insulin release. Curcumin also decreased β-cell volume, presumably reflecting loss of Cl^- (and hence water) as a result of anion channel activation. These findings are consistent with the suggestion that Cl^- fluxes play an important role in regulating β-cell function. The stimulation of β-cell function by curcumin could contribute to the hypoglycaemic actions of this compound, and these findings identify a novel potential therapeutic target for the treatment of type 2 diabetes mellitus.

1. Introduction

Curcumin (1,7-bis-[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) is a polyphenolic compound forming a major component of the spice turmeric. It has been used extensively as a traditional medicine, notably on the Indian subcontinent, and has been ascribed numerous therapeutic properties, including anti-inflammatory, anti-oxidant, anti-cancer and anti-microbial activity (see [1] for review). In addition, a number of studies have reported an antidiabetic action of curcumin. For example, administration of curcumin was shown to reduce blood glucose levels in alloxan- and streptozotocin-diabetic rats [2,3] and genetically diabetic KK-Ay mice [4].

In accordance with its diverse actions, curcumin has been shown to exert numerous effects at the molecular level. These include inhibition of cyclooxygenase-2 expression [5], stimulation of the peroxysome proliferator-activated receptor γ (PPAR-γ) [6], inhibition of NAD(P)H:quinone oxidoreductase 1 [7] and of the inositol 1,4,5-trisphosphate receptor [8]. Curcumin has also been reported to increase permeability of the mitochondrial membrane by opening the permeability transition pore [9]. Of particular therapeutic interest, curcumin was reported to correct cystic fibrosis defects via an action

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Abbreviations: NPPB, 5-nitro-2-(3-phenylpropylamino) benzoic acid; VRAC, volume-regulated anion channel

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on trafficking of the misfolded mutant cystic fibrosis transmembrane conductance regulator (CFTR) ΔF508 channel, thereby inducing the functional appearance of the channel in the plasma membrane [10]. However, subsequent studies did not support this suggestion [11–13]. A possible explanation for this discrepancy was provided by a recent report showing a direct stimulatory effect of curcumin on the CFTR channel [14,15], possibly involving interaction with the nucleotide-binding domain of the channel [14].

The action of curcumin on CFTR led us to investigate whether curcumin might influence the activity of another chloride channel, the volume-regulated anion channel (VRAC) [16,17] in pancreatic β-cells. There is increasing evidence that this channel plays an important role in regulating electrical and secretory activity in the β-cell. For example, both glucose and sulphonylureas have been shown to activate the channel, generating an inward (depolarising) current due to Cl^- efflux [16,18,19]. Correspondingly, electrical activity and insulin release in response to glucose or sulphonylureas are sensitive to inhibition by all VRAC blockers so far studied [20–23]. To date, no pharmacological activators of the VRAC have been described. The identification of such a compound would be valuable in further elucidating the role of the VRAC in regulating β-cell function and could also lead to the identification of a potential novel drug target for the treatment of diabetes mellitus.

2. Methods and materials

2.1. Islet and β-cell preparation

Pancreatic islets were isolated from fed Sprague–Dawley rats (300–350 g; either sex) by collagenase digestion. The standard incubation medium used for islet cell preparation and incubations consisted of (mM) 120 NaCl, 5 KCl, 1 $MgSO_4$, 1 NaH_2PO_4, 1.2 $CaCl_2$, 25 Hepes–NaOH (pH 7.4) and 4 glucose. Islets were dispersed into single cells by mixing briefly in a nominally Ca^{2+}-free medium consisting of 120 NaCl, 5 KCl, 2 $MgSO_4$, 4 glucose, 1 EGTA, 1% (w/v) bovine serum albumin (Boehringer, fraction V) and 25 Hepes–NaOH (pH 7.4). Dispersed cells were centrifuged at 100 g for 5 min and re-suspended in Hepes–buffered minimal essential medium (MEM; Gibco, Paisley, Scotland). Aliquots of cell suspension were plated onto 30 mm diameter polystyrene dishes (Nunc) and cultured for 3–12 days in humidified air at 37 °C. β-Cells were identified on the basis of their size (larger than non β-cells), their characteristic granular appearance and polarised membrane potential (~ −60 mV) in the presence of 'fasting' concentrations of blood glucose. We have previously shown that cells identified by such criteria correspond to insulin-staining cells [24].

2.2. Electrophysiological experiments

Cells were superfused at a rate of approximately 2 ml/min with incubation medium. β-Cell membrane potential, whole-cell and single channel currents were recorded with a List EPC-7 amplifier (List, Darmstadt, Germany). Activity of the VRAC was assessed at the whole-cell and single channel levels. First, cells were voltage-clamped at 0 mV in the perforated patch configuration, with a pipette solution containing 140 CsCl, 1 $MgSO_4$, 10 HEPES–CsOH (pH 7.2) and 50 μg/ml gramicidin, which preserves intracellular [Cl^-], as perforating agent [25]. In certain experiments, the Cl^-—permeable amphotericin was used as perforating agent in combination with a pipette solution containing 6 mM Cl^- (gluconate as substituent) in order to lower intracellular [Cl^-] [25]. Current responses to 50 ms pulses of ±100 mV at 2 s intervals were recorded. *I/V* curves were constructed by subjecting cells to 500 ms voltage pulses from −100 to +100 mV in 20 mV increments. No subtraction of leak current was carried out in either case. VRAC activation is manifest as a characteristic outwardly rectifying current [16,17]. Single channel recordings of VRAC activity were made using the cell-attached configuration of the patch–clamp technique [18,19]. The pipette solution consisted of 140 CsCl, 1 $MgCl_2$ and 10 Hepes–CsOH (pH 7.4). At a pipette potential of 0 mV, channel openings generated an inward current recorded as downward deflections. Current recordings were filtered at 30 Hz and stored for subsequent analysis. Channel activity is expressed as *NP*, where *N* is the number of channels (or conductance states) and *P* is open probability. This value was calculated from current–amplitude histograms constructed from 60 s segments of recording and fitted to Gaussian distributions by the 'least squares' method using pClamp6 software. Input conductance (G_{input}) has been used as an index of K_{ATP} channel activity [25,26] and was measured by subjecting cells to 50 ms pulses of ±10 mV at 2 s intervals from a holding potential of −65 mV. The pipette solution consisted of 140 KCl, 10 NaCl, 10 Hepes–NaOH (pH 7.2) and 50 μg/ml gramicidin. Membrane potential was recorded under current clamp conditions (zero current) with the same pipette solution.

2.3. Cell volume measurements

Changes in β-cell volume were measured by a video-imaging technique as described previously [17,27]. Cell volume is expressed as relative cell volume (i.e. normalised with respect to the volume observed for the initial reading which is given as 1.00). Statistical analysis was made by comparing areas under each curve for test and control cells during the period of exposure to curcumin (3–13 min), using unpaired *t*-test. All measurements were carried out 'blind' in order to avoid bias.

2.4. Insulin secretion

The effects of curcumin on insulin release were studied using groups of 10 intact islets incubated in 1 ml. medium. Following a 30 min incubation, samples of supernatant were removed and the insulin content measured by radioimmunoassay.

Collagenase (type 4) was obtained from Worthington (Cambridge Biosciences, Cambridge, UK). Curcumin and all other chemicals were supplied by the Sigma Chemical Co., Poole, UK. In all experiments, a stock solution of curcumin (10 mM in DMSO) was made up immediately prior to each experiment, and added to the incubation medium at the appropriate dilution. ^{125}I-insulin was obtained from Diagnostic Systems Laboratories, Oxfordshire, UK.

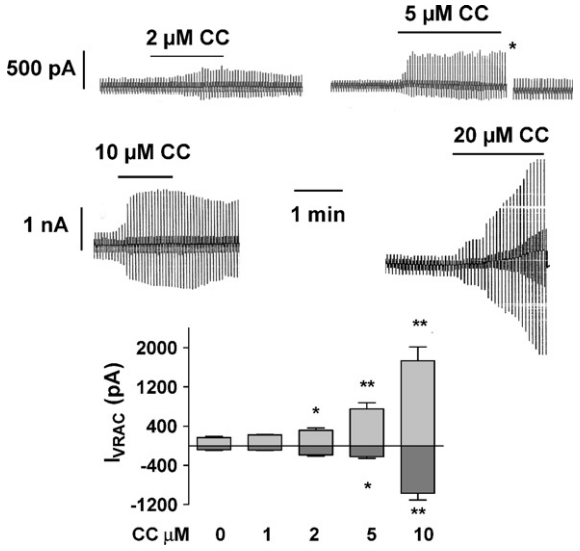

Fig. 1 – Activation of the VRAC in rat pancreatic β-cells by curcumin (CC); perforated patch recording of whole-cell currents. A Cs^+-rich pipette solution was used to block K^+ currents containing 50 μg/ml gramicidin. Cells were held at 0 mV and subjected to 50 ms pulses of ±100 mV. The gap in the top right hand trace marked * represents a period of 12 min following withdrawal of curcumin. (Bottom right panel) Mean (±S.E.) values of outward (+ve) and inward (−ve) currents evoked by different concentrations of curcumin from 5 to 10 cells. $^{**}p < 0.005$; $^{*}p < 0.01$ by paired *t*-test.

3. Results

3.1. *Effects of curcumin on VRAC and electrical activity*

The application of curcumin to rat pancreatic β-cells voltage-clamped under perforated patch conditions caused a concentration-dependent activation an outwardly rectifying current (Figs. 1 and 2). Current reversal occurred at a potential of -22.2 ± 1.3 mV (with 5 μM curcumin; $n = 6$). These characteristics of the whole-cell current are consistent with those of the VRAC activated by hypotonic cell swelling [16–18,28]. The lowest effective concentration of curcumin was 2 μM, no significant activation of the VRAC being found with 1 μM curcumin, even following prolonged exposure for up to 30 min. Reversal of VRAC activation occurred with a considerable delay following withdrawal of the compound and with the higher concentration of 10 μM, no reversal of activation was observed even 30 min or more following washout. When applied at the higher concentration of 20 μM, curcumin caused a rapid, progressive and irreversible increase in membrane conductance. The associated 'leak' current reversed close to 0 mV and we suggest that this phenomenon represents a non-specific disruption of the integrity of the plasma membrane.

Activation of the VRAC by curcumin was next studied at the single-channel level using the cell-attached configuration. Channel activation by curcumin under such conditions is shown in Fig. 3. At pipette potentials of 0 mV (Fig. 3A) and −80 mV (Fig. 3B), channel openings generated inward and outward currents, respectively. Current reversal generally occurred between −20 and −40 mV which is likely to be close to the membrane potential in the presence of curcumin. Again, a current with essentially similar characteristics has been previously reported in rat β-cells in response to both hypotonic cell swelling and glucose stimulation, and is thought to represent the VRAC [18]. Activation of the channel by curcumin was manifest as an increase in open channel probability (NP), the values for which were 0.10 ± 0.03 and 0.46 ± 0.10 in the absence and presence of curcumin, respectively ($n = 6$, $p < 0.02$ by paired *t*-test).

We and others have previously shown that VRAC activation in rat β-cells by hypotonic cell swelling causes depolarisation and electrical activity [28,29]. The next series of experiments therefore examined the effects of curcumin on β-cell membrane potential, again using the perforated patch recording technique. As shown in Fig. 4, application of curcumin over the concentration range 2–10 μM in the presence of 4 mM glucose depolarised the membrane potential and evoked electrical activity. As with VRAC activation, the magnitude of this effect depended upon the concentration of curcumin, with little or no effect on membrane potential apparent at 1 μM. In addition, some reversibility was usually apparent with 2 or 5 μM curcumin whereas with 10 μM, a progressive and sustained depolarisation was usually observed. In the absence of glucose, β-cell resting membrane potential was considerably hyperpolarised (-72.4 ± 1.4 mV, $n = 5$), presumably due to activation of K_{ATP} channels. Under such conditions, application of 5 μM curcumin depolarised the cells to -61.0 ± 3.2 mV ($p < 0.02$

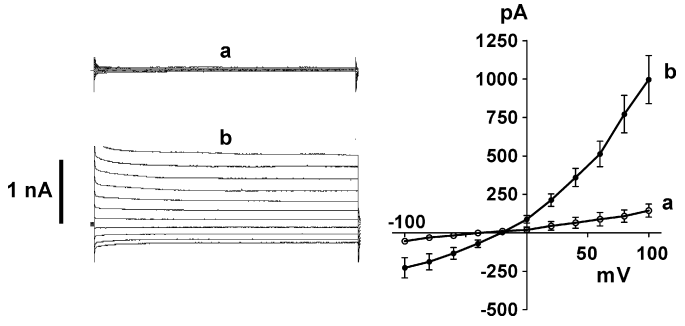

Fig. 2 – Current–voltage relationship in the absence (a) and presence (b) of 5 μM curcumin. Pipette and bath solutions as in Fig. 1. Cells were exposed to 500 ms voltage pulses from −100 to +100 mV with 20 mV increments. *I/V* plots are mean (±S.E.) derived from four and six cells, respectively.

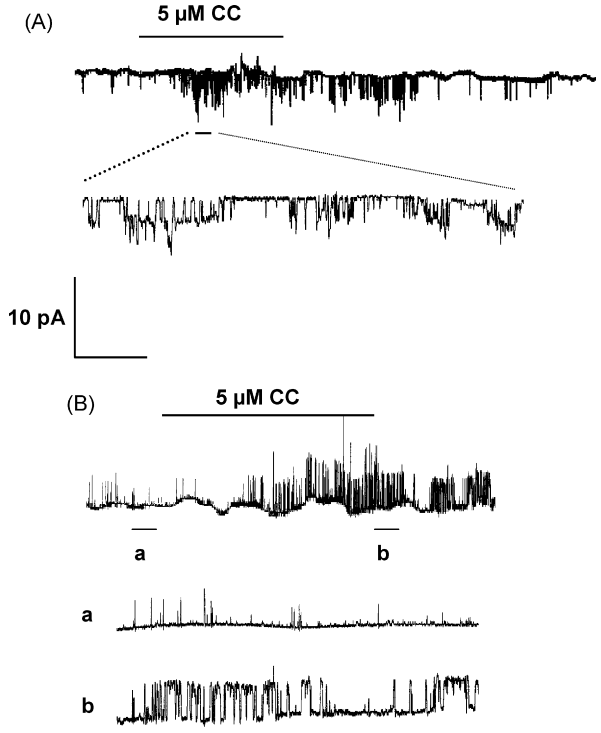

Fig. 3 – Cell-attached recordings of VRAC activity in rat pancreatic β-cells. (A) Effect of curcumin (CC; 5 μM) in the presence of 6 mM glucose. A Cs^+-rich pipette solution was used with a holding potential of 0 mV. Downward deflections represent inward currents. (B) Recording of outward current, shown as upward deflections, at a holding potential of −80 mV. Horizontal calibration bar: 1 min (main traces) or 3 s (expanded traces).

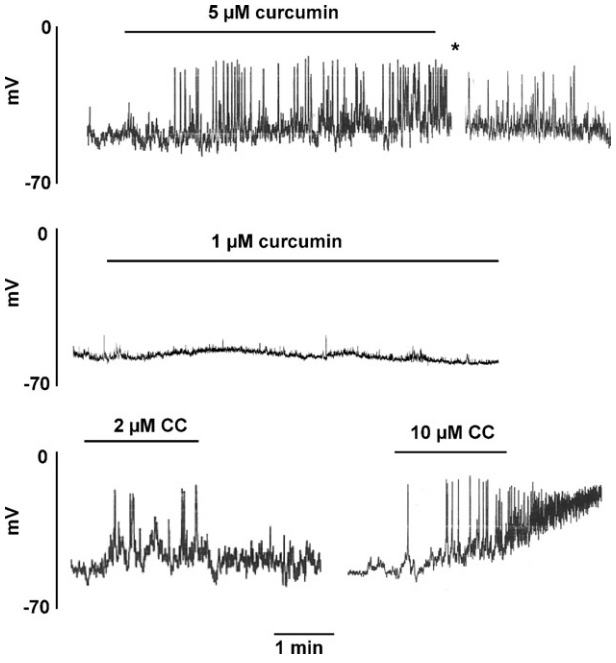

Fig. 4 – Depolarisation of membrane potential and induction of electrical activity in rat pancreatic β-cells by curcumin (CC; 1, 2, 5 and 10 μM). Membrane potential was recorded using the perforated patch technique with a K^+-rich pipette solution containing 50 μg/ml gramicidin and a bath solution containing 4 mM glucose. The gap in the upper trace * represents a period of 10 min following withdrawal of curcumin. The traces are representative of those from 4 to 10 recordings.

by paired *t*-test), though this was not sufficient to evoke electrical activity (not shown).

Activation of the β-cell VRAC current, either by hypotonic cell swelling or by glucose stimulation, is sensitive to inhibition by a number of anion channel blockers, notably 5-nitro-2-(3-phenylpropylamino) benzoic acid [17,20]. As shown in Fig. 5, activation of the VRAC current and the induction of electrical activity by curcumin were also sensitive to inhibition by 50 μM NPPB. This finding suggests that the current activated by curcumin is the VRAC, and not due to an increase in non-specific leak current.

Recent studies from this laboratory have shown that β-cell activation by glucose depends on raised intracellular $[Cl^-]$ [25], possibly because VRAC activation by glucose will only generate an inward (depolarising) current when E_{Cl} is positive with respect to the resting membrane potential. As shown in Fig. 6, β-cell depolarisation and electrical activity in response to curcumin was also dependent upon intracellular $[Cl^-]$. Thus, no depolarisation was seen when intracellular $[Cl^-]$ was reduced by the use of the Cl^-—permeable perforating agent amphotericin in combination with a pipette solution containg 6 mM Cl^- (Fig. 6A) or by inhibition of the $Na^+/K^+/2Cl^-$ (NKCC1) transporter with bumetanide (Fig. 6B). Indeed, under such conditions, curcumin evoked a modest hyperpolarisation in 2/4 cells, presumably due to Cl^- entry following VRAC activation. In both cases, a marked depolarisation could be induced by 25 mM K^+ under these conditions. Essentially similar findings were previously obtained using glucose as a stimulus [25], consistent with the suggestion that curcumin, like glucose, acts on the β-cell predominantly by activating the VRAC and inducing an inward Cl^- current.

3.2. *Input conductance*

Since the β-cell membrane potential is subject to regulation by changes in K_{ATP} channel activity, experiments were carried out to investigate whether curcumin exerted any influence on whole-cell input conductance (G_{input}), to which K_{ATP} channel activity makes a major contribution [26]. As shown in Fig. 7, the application of 5 μM curcumin caused a significant increase in G_{input} from a value of 1.14 ± 0.26 nS in the sole presence of 4 mM glucose to 1.71 ± 0.32 nS after the addition of curcumin ($n = 6$; $P < 0.05$ by paired *t*-test). As apparent from the expanded traces, this effect was accompanied by an increase in current noise. Both phenomena presumably reflected, at least in part, activation of the VRAC. Thus, there was no evidence of K_{ATP} channel inhibition by curcumin, though we cannot exclude the possibility of activation of an inward current in addition to the VRAC.

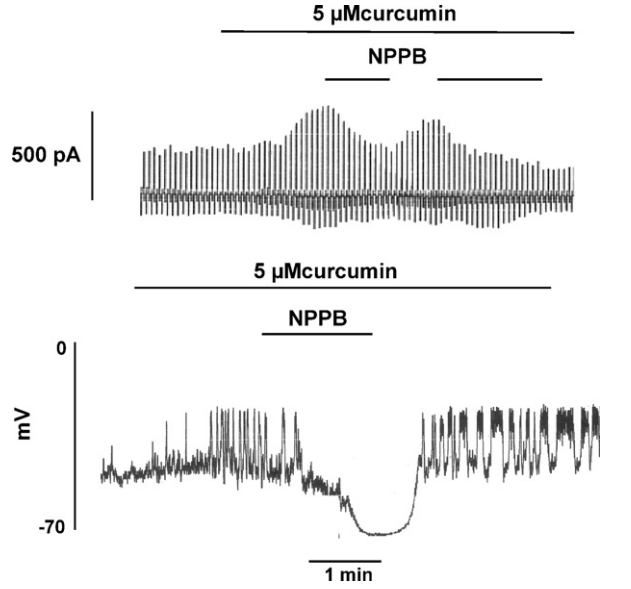

Fig. 5 – Inhibition of curcumin-induced VRAC (*upper panel*) and electrical activity (*lower panel*) by 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB; 50 μM). The experimental conditions were as outlined in Figs. 1 and 4, respectively, similar recordings being obtained from three cells.

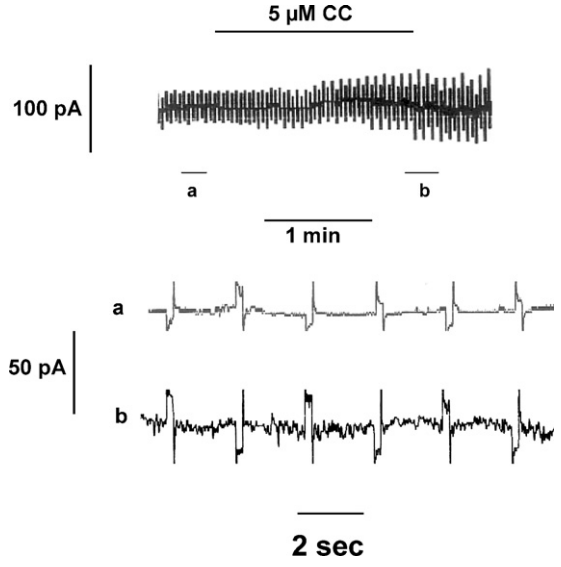

Fig. 7 – Effect of curcumin (CC; 5 μM) on input conductance in a rat pancreatic β-cell; perforated patch recording. The K^+-rich pipette solution contained 50 μg/ml gramicidin and the bath solution 4 mM glucose. Cells were held at −65 mV and subjected to 50 ms pulses of ±10 mV. The expanded traces and show currents in the absence (a) and presence (b) of curcumin.

3.3. *β-Cell volume*

As noted above, activation of the VRAC by curcumin generates an inward (depolarising) current due to Cl^- efflux. Such an effect might be expected to be accompanied by K^+ and water loss and a consequent reduction in cell volume. Experiments were therefore carried out to study the effects of curcumin on relative cell volume (RCV; Fig. 8). During the 3 min period prior to the application of curcumin, mean RCV (relative to time zero) was 1.01 ± 0.003 ($n = 5$). The addition of 5 μM curcumin caused a gradual reduction in RCV to a minimum of 0.93 ± 0.02 during the period 12–13 min. Analysis of the areas under the curve for each cell indicated that this effect of curcumin was statistically significant ($p < 0.02$). Withdrawal of curcumin was followed by an apparent partial recovery of RCV. No significant changes in RCV were seen in control cells where no additions were made.

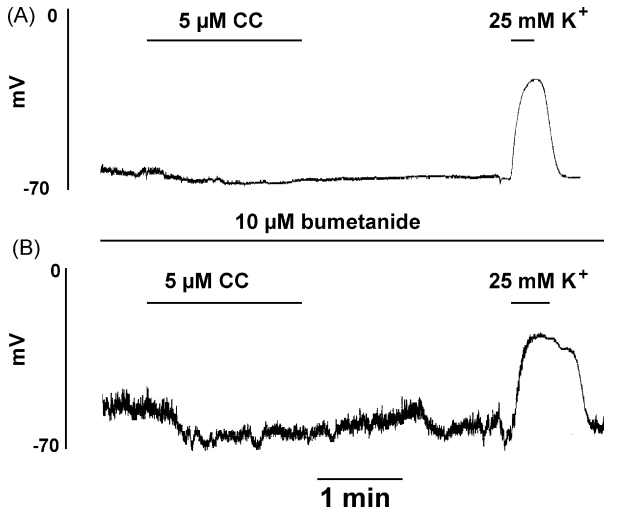

Fig. 6 – Curcumin (CC; 5 μM) does not induced electrical activity when intracellular [Cl^-] is reduced. (A) Intracellular [Cl^-] was reduced by the use of amphotericin as perforating agent in combination with a pipette solution containing 6 mM Cl^-. (B) Gramicidin-perforated patch recording; intracellular [Cl^-] was reduced by inhibition of NKCC1 with 10 μM bumetanide. Both recordings are representative of those from four cells.

3.4. *Insulin release*

The induction of β-cell electrical activity by curcumin would be expected to lead to enhanced insulin release. As shown in Fig. 9, concentrations of curcumin over the range 2–10 μM caused a significant stimulation of insulin release from intact islets. At the higher concentration of 20 μM, curcumin had no apparent effect on insulin release, possibly reflecting an unidentified inhibitory action of the drug at higher concentrations. The stimulatory effect of curcumin on insulin release occurred over the same concentration range as for electrical activity, although the concentration-dependency of this insulinotropic action was less clear than for electrical activity. However, it should be borne in mind that insulin release was measured using freshly prepared intact islets in batch incubations, as opposed to cultured cells used for the more dynamic electrophysiological experiments. The stimulation of insulin release by 5 μM curcumin was sensitive to inhibition by the VRAC inhibitor NPPB and was dependent upon the presence of glucose in the incubation medium, consistent

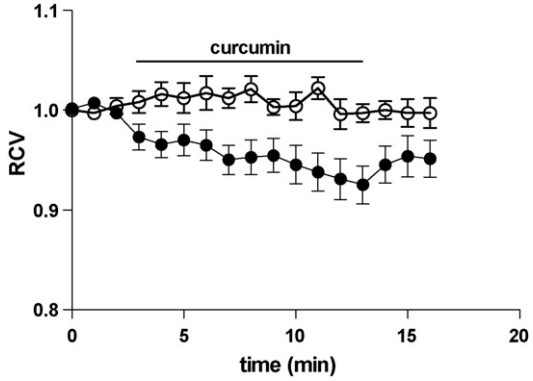

Fig. 8 – Effect of curcumin (5 μM; solid symbols) on relative cell volume (RCV) in rat pancreatic β-cells in the presence of 4 mM glucose. The open symbols represent control cells where no additions were made. In the former case, curcumin was applied for the period shown by the horizontal bar. Each point represents the mean ± S.E.M. from five and four cells, respectively.

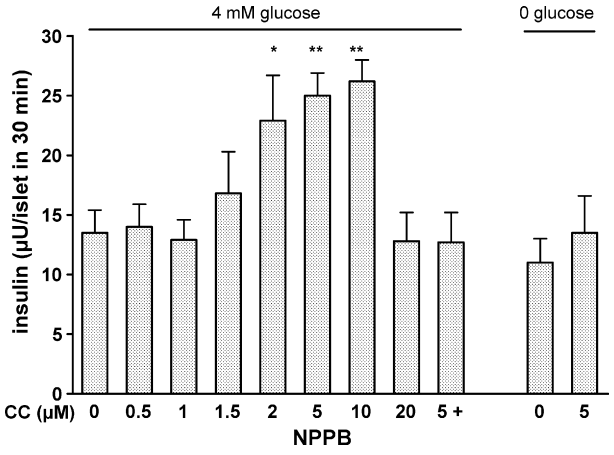

Fig. 9 – Effect of curcumin (CC) on insulin release from intact pancreatic islets. Groups of 10 islets were incubated for 30 min in the absence or presence of glucose (4 mM) and NPPB (50 μM). The data are means ± S.E.M. of six to eight replicates. *P < 0.02; **P < 0.01.

with membrane potential recordings under comparable conditions.

4. Discussion

Among the numerous reported therapeutic effects of curcumin is a hypoglycaemic action in experimental diabetes mellitus [2–4]. The mechanism(s) underlying this effect have not yet been fully explored, but could involve activation of PPARγ [4,6], the site of action of the thiazolidinedione class of antidiabetic drugs [30]. Whilst this latter action would suggest an extra-pancreatic effect of curcumin on blood glucose levels, the present study demonstrates that curcumin also exerts a direct stimulatory effect on pancreatic β-cell function. The stimulation of β-cells by curcumin was associated with depolarisation, electrical activity and insulin release. It is likely that these events are causally linked. First, the above responses were all evoked by curcumin over a similar, if rather narrow, concentration range and with similar kinetics. Second, all responses to curcumin were sensitive to inhibition by the anion channel blocker NPPB at a concentration previously shown not to affect basal insulin release [20], although it should be borne in mind that this compound is unlikely to be a selective VRAC inhibitor. It is still conceivable that curcumin could exert additional effects on insulin release independent of changes in membrane potential. Indeed, at high concentrations, curcumin appeared to cause irreversible damage to the plasma membrane, an effect associated with impaired insulin release. The nature of this deleterious action of curcumin remains to be established.

The depolarisation evoked by curcumin appeared to be the result of activation of an anion channel, leading to Cl^- efflux. The characteristics of the curcumin-activated channel bear a striking resemblance to those of the VRAC at both whole-cell and single channel levels. The mechanism of VRAC activation by curcumin is at present unknown, although the finding that the compound causes cell shrinkage, rather than swelling indicates that channel activation is not a secondary response to increased cell volume, but is likely to be due to an interaction of the compound with the channel protein. As noted earlier, a direct stimulatory effect of curcumin has been reported on the CFTR anion channel [14,15], possibly involving interaction with the nucleotide-binding domain of the channel [14]. It has been shown that the VRAC is subject to regulation by nucleotides [16,17,31] raising the possibility that curcumin might interact with a similar, putative nucleotide-binding domain on the VRAC. It should be pointed out that the molecular identity and structure of the VRAC channel protein remain to be established in any cell type.

An alternative possibility is that curcumin activates the VRAC indirectly, via hydrophobic interaction with plasma membrane lipids. In this regard, it has been suggested that changes in tension in the cell membrane could be important in modulating volume-regulated anion channels [32]. The highly lipophilic nature of curcumin was evident from our own experiments where a clear yellow staining was retained by β-cells after exposure to the drug at higher concentrations, even after a prolonged period of 'washout'. This probably explains the slow reversibility of curcumin-induced activation following washout.

The findings of the present study have clear implications regarding the role of the VRAC in regulating β-cell function. Activation of this channel by exposure to hypotonic solutions generates an inward depolarising current due to Cl^- efflux. This occurs because intracellular $[Cl^-]$ is maintained above its electrochemical equilibrium through the activity of the $Na^+/K^+/2Cl^-$ co-transporter NKCC1 [24]. Depolarisation of the plasma membrane potential leads to electrical activity and insulin release [28,29]. As noted earlier, there is also increasing evidence that activation of this channel plays an important role in coupling increased glucose metabolism to the induction of electrical activity in the β-cell (see [33] for review). Thus, a rise in glucose concentration activates the VRAC [18,20,34,35]

involving increased glucose metabolism and possibly increased β-cell volume [27,35]. This action probably explains the stimulation of $^{36}Cl^-$ [36,37] and ^{3}H-taurine [38,39] efflux by glucose and increased Cl^- permeability [40]. The results of the present study suggest that the direct activation of the VRAC by curcumin also leads to Cl^- efflux, thereby generating an inward current, depolarisation and electrical activity and are thus consistent with a role for the VRAC in regulating electrical and secretory activity in the β-cell.

The concentration range of curcumin effective in causing β-cell activation (2–10 μM) considerably exceeds the low nanomolar concentrations measured in plasma during oral administration of the compound [1]. However, the strongly lipophilic nature of curcumin noted earlier could result in its accumulation to considerably higher levels in tissues with prolonged exposure. Thus our findings could explain, at least in part, the hypoglycaemic action of curcumin. This would have potential therapeutic implications in identifying the β-cell VRAC as a potential novel drug target in the treatment of type 2 diabetes mellitus.

Acknowledgements

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