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The comprehensive electrophysiological study of curcuminoids on delayed-rectifier K^+ currents in insulin-secreting cells

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ABSTRACT

Curcumin (CUR) has been demonstrated to induce insulin release from pancreatic β-cells; however, how curcuminoids (including demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC)) exert any possible effects on membrane ion currents inherently in insulin-secreting cells remains largely unclear. The effects of CUR and other structurally similar curcuminoids on ion currents in rat insulin-secreting (INS-1) insulinoma cells were therefore investigated in this study. The effects of these compounds on ionic currents and membrane potential were studied by patch-clamp technique. CUR suppressed the amplitude of delayed-rectifier K^+ current ($I_{K(DR)}$) in a time-, state- and concentration-dependent manner in these cells and the inhibition was not reversed by diazoxide, nicorandil or chlorotoxin. The value of dissociation constant for CUR-induced suppression of $I_{K(DR)}$ in INS-1 cells was 1.26 μM. Despite the inability of CUR to alter the activation rate of $I_{K(DR)}$, it accelerated current inactivation elicited by membrane depolarization. Increasing CUR concentrations shifted the inactivation curve of $I_{K(DR)}$ to hyperpolarized potential and slowed the recovery of $I_{K(DR)}$ inactivation. CUR, DMC, and BDMC all exerted depressant actions on $I_{K(DR)}$ amplitude to a similar magnitude, although DMC and BDMC did not increase current inactivation clearly. CUR slightly suppressed the peak amplitude of voltage-gated Na^+ current. CUR, DMC and BDMC depolarized the resting potential and increased firing frequency of action potentials. The CUR-mediated decrease of $I_{K(DR)}$ and the increase of current inactivation also occurred in βTC-6 INS-1 cells. Taken these results together, these effects may be one of the possible mechanisms contributing their insulin-releasing effect.

1. Introduction

Curcumin (CUR, diferuloylmethane, (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6- heptadiene-3,5-dione), a major constituent of the spice turmeric, is a bright yellow diarylheptanoid produced by some plants. It is the principal curcuminoid of turmeric, which is a member of the ginger family (*Zingiberaceae*). This compound is sold as an herbal supplement, cosmetics ingredient, food flavoring and food coloring (Priyadarsini, 2014). Particularly, this nutraceutical compound has been demonstrated to possess therapeutic properties against a variety of diseases ranging from cancer to diabetes mellitus (Kiuchi et al., 1993; El-Azab et al., 2011; Chuengsamarn et al., 2012; Guo et al., 2013; Meng et al., 2013; Castro et al., 2014; Ghorbani et al., 2014; Priyadarsini, 2014; Jiménez-Osorio et al., 2016; Weisberg et al., 2016). For example, CUR has been recently reported to influence insulin release from isolated pancreatic islets (Abdel Aziz et al., 2010, 2014; Ghorbani et al., 2014; Rouse et al., 2014).

There are growing evidences to show that CUR and other curcuminoids might directly perturb membrane ion channels. For example, it has been demonstrated that CUR could block *erg* K^+ currents seen in monocytic leukemia cells (Banderali et al., 2011) and $K_V1.4$-encoded current (Liu et al., 2006). This compound was recently reported to enhance the activity of large-conductance Ca^{2+} -activated K^+ (BK_{Ca}) channels (Chen et al., 2015) and to activate volume-regulated anion channel (Best et al., 2007). It also suppressed voltage-gated K^+ currents in rabbit coronary arterial smooth myocytes (Hong et al., 2013) and Na^+ current in dorsal root ganglion neurons (Meng et al., 2015). The inactivation of $K_V2.1$ channels in response to membrane depolarization can be modulated by the presence of CUR (Aréchiga-Figueroa et al., 2015). Although CUR and curcuminoids can disturb the function of

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pancreatic β-cells (Best et al., 2007; Abdel Aziz et al., 2010, 2014; Meng et al., 2013; Rouse et al., 2014), their effects on ion currents in these cells have not been thoroughly studied.

The insulin-secreting (INS-1) cell line, one of the widely used insulin-secreting cell lines, was originally generated from irradiated primary pancreatic β-cells (Skelin et al., 2010). Despite being transformed and requiring β-mercaptoethanol which is toxic and irritating (Asfari et al., 1992), INS-1 cells have retained some characteristics of normal pancreatic β-cells. These cells were reported to have insulin content, and to be responsive to glucose and associated with expression of GLUT-2 and glucokinase. They have indeed attracted growing interest as a suitable model for investigating the cellular mechanisms of how insulin secretion can be regulated (Asfari et al., 1992; Skelin et al., 2010; Chen et al., 2011; Song et al., 2015; Kittl et al., 2016). Therefore, in this study, we intended to extract CUR and curcuminoids (including demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC)) to investigate the possible effects of those compounds on ion currents and membrane potential in rat INS-1 insulinoma cells by assessing its effects on the magnitude and kinetics of $I_{K(DR)}$.

2. Materials and methods

2.1. General experimental procedures

All the solvents used were purchased from Merck KGaA (Darmstadt, Germany). Melting points were determined using a Yanagimoto MP-S3 apparatus. The UV spectra were obtained on a Hitachi UV-3210 spectrophotometer, and IR spectra were recorded on a Shimadzu FTIR-8501 spectrophotometer. ^{1}H- and ^{13}C NMR spectra were obtained on the Bruker Avance III-400 NMR spectrometer with tetramethylsilane as internal standard, and the chemical shifts were reported in δ values (ppm). Column chromatography was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, E. Merck).

2.2. Extraction and fractionation of medicinal plants

Medicinal plant materials were purchased in the market and authenticated by Dr. T. F. Hsieh (Floriculture Research Center, Taiwan Agricultural Research Institute, Yunlin, Taiwan) and the voucher specimens (PCKuo_20100101) were deposited in the herbarium of School of Pharmacy, National Cheng Kung University, Tainan City, Taiwan. The dried materials of *Curcuma longa* Linn'e (180 g) were extracted with methanol under reflux (10 L × 8 × 8 h), and the crude extracts were concentrated in vacuo to give a brown syrup (15 g). The crude extract was partitioned between ethyl acetate and water to afford ethyl acetate solubles (13 g) and water extracts (2 g), respectively. The ethyl-acetate soluble fraction was applied to a silica gel column, and then eluted with chloroform and step gradient of acetone (300:1, 100:1, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1, respectively) and monitored by total liquid chromatography to yield eleven fractions (Frs. 1–11). Fr. 3 was subjected to column chromatography on silica gel, eluted with *n*-hexane–acetone step gradient system (6:1, 3:1, and 1:1, respectively), to afford five minor fractions (Frs. 3.1–3.5). Fr. 3.5 was further recrystallized with *n*-hexane and ethyl acetate to afford CUR (**1**) (100 mg) (Jayaprakasha et al., 2002). Fr. 6 was purified by silica gel column chromatography eluted with *n*-hexane/ethyl acetate step gradient system (4:1, 2:1, and 1:1, respectively) to yield eight minor fractions (Frs. 6.1–6.8). Fr. 6.5 was further purified by silica gel column chromatography eluted with chloroform and step gradient of ethyl acetate (300:1, 100:1, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1, respectively) to produce six subfractions (Frs. 6.5.1–6.5.6). Frs. 6.5.2 and 6.5.5 were recrystallized with *n*-hexane and ethyl acetate to afford DMC (**2**) (15 mg) and BDMC (**3**) (18 mg), respectively (Jayaprakasha et al., 2002). The chemical structures of CUR, DMC and BDMC are illustrated in Fig. 1. Their purities were better than 99.4% as determined by HPLC.

2.3. Cell preparation

The rat INS-1 cell line (clone 832/13) was kindly provided by Dr. Christopher B. Newgard, Duke University, Durham, NC, USA. Cells were plated in 10-cm plate and cultured in RPMI-1640 medium with 11.1 mM D-glucose (Invitrogen) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, and 50 μM β-mercaptoethanol (Chen et al., 2011). The clonal strain βTC-6 cell line was obtained from the American Type Culture Collection ([CRL-11506], Manassas, VA). This cell line was derived from a pancreatic tumor (insulinoma) arising in a transgenic mouse. βTC-6 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 4 mM L-glutamine, 4.5 g/L glucose, 1.5 g/L sodium bicarbonate, and 15% fetal bovine serum. INS-1 or βTC-6 cells were maintained at 37 °C in a 95% air and 5% CO_2 humidified atmosphere. The viability of these cells during cell exposure to different concentrations of CUR was assessed by the trypan blue-exclusion test. The experiments were commonly made five or six days after cells had been cultured (60–80% confluence).

2.4. Electrophysiological measurements

Shortly before experiments were made, cells were dissociated and an aliquot of cell suspension was transferred to a home-made recording chamber mounted on the stage of an inverted CKX-41 microscope (Olympus, Tokyo, Japan). Cells were bathed at room temperature in normal Tyrode's solution (in mM): 136.5 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.53 $MgCl_2$, 5.5 glucose, and 5.5 HEPES-NaOH buffer (pH 7.4). Patch pipettes were made of Kimax-51 capillaries (Kimble, Vineland, NJ) using a vertical PP-830 puller (Narishige, Tokyo, Japan) and their tips were fire-polished with an MF-83 microforge (Narishige). The pipettes used had a tip resistance of 3–5 MΩ when immersed in normal Tyrode's solution. Current recordings were made using the patch-clamp technique in either whole-cell, cell-attached, or inside-out configuration with an RK-400 amplifier (Bio-Logic, Claix, France) (Lo et al., 2015; So et al., 2017). All liquid junction potentials, which develop at the electrode tip when the composition of the internal solution differed from that in the bath, were corrected before the seal was made.

2.5. Data recordings

The signals, consisting of voltage and current tracings, were stored online in a laptop computer at the sampling rate of 10 kHz through a Digidata-1322A acquisition system (Molecular Devices, Sunnyvale, CA), which was equipped with an SCSI card (SlimSCSI 1460; Adaptec Milpitec, CA) and pCLAMP 9.2 software (Molecular Devices). Current signals were low-pass filtered at 1 or 3 kHz. In some experiments, the linear passive leak currents were digitally subtracted using a P/4 regimen. By use of digital-to-analog conversion, the pCLAMP-created profiles for either rectangular or ramp-voltage pulses were used to determine either current-voltage (*I-V*) relationships or steady-state inactivation of ionic currents (e.g., $I_{K(DR)}$). For further analyses, the digital data were exported to either graphing software (OriginPro 2016; OriginLab, Northampton, MA) or a spreadsheet under Excel 2013 (Microsoft, Redmond, WA).

2.6. Data analysis

To calculate percentage inhibition of CUR, DMC or BDMC on $I_{K(DR)}$, each cell was depolarized from − 50 to + 50 mV with a duration of 1 s. Current amplitudes measured at the end of each depolarizing pulse in the presence of these compounds were compared with the control value (i.e., in the absence of any agent). The concentration-dependent relationships of CUR and curcuminoids on the inhibition of $I_{K(DR)}$ amplitude were fitted to a modified Hill function by nonlinear least-squares regression analysis with OriginPro 2016 (OriginLab); that is,