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Curcumin potently blocks Kv1.4 potassium channels

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Abstract

Curcumin, a major constituent of the spice turmeric, is a nutriceutical compound reported to possess therapeutic properties against a variety of diseases ranging from cancer to cystic fibrosis. In whole-cell patch-clamp experiments on bovine adrenal zona fasciculate (AZF) cells, curcumin reversibly inhibited the Kv1.4 K^+ current with an IC_{50} of 4.4 μM and a Hill coefficient of 2.32. Inhibition by curcumin was significantly enhanced by repeated depolarization; however, this agent did not alter the voltage-dependence of steady-state inactivation. Kv1.4 is the first voltage-gated ion channel demonstrated to be inhibited by curcumin. Furthermore, these results identify curcumin as one of the most potent antagonists of these K^+ channels identified thus far. It remains to be seen whether any of the therapeutic actions of curcumin might originate with its ability to inhibit Kv1.4 or other voltage-gated K^+ channel.

Keywords

Curcumin; Potassium channel; Kv1.4

Curcumin (diferuloylmethane) is a phenolic compound isolated from the rhizome of *Curcuma longa* (turmeric), commonly used as a spice in some cultures where daily intake may reach several grams per day. Curcumin is also a nutriceutical agent reported to have therapeutic activity against a variety of diseases including cancer, Alzheimer's disease, and cystic fibrosis [1–5]. At the molecular level, curcumin produces a range of effects, some of which may underlie its putative therapeutic actions [6]. The anti-cancer actions of curcumin may be due to its irreversible inhibition of amino-peptidase N1, an enzyme that plays a key role in tumor invasion and angiogenesis [1]. Curcumin induces apoptosis in mammary tumor cells through an action on P53 expression [2]. The potential effectiveness of curcumin in the prevention of Alzheimer's disease may originate with its ability to inhibit formation of amyloid β oligomers and fibrils through direct binding to β amyloid species [3]. Well-known antioxidant and anti-inflammatory actions of curcumin may underlie its ability to induce heme oxygenase, a protein that provides efficient protection against oxidative stress [7].

More recently, curcumin has been shown to correct cystic fibrosis defects through its actions on the cystic fibrosis transmembrane conductance Cl-channel [4]. In cystic fibrosis, the cystic fibrosis transmembrane conductance regulator (CFTR) is retained in the endoplasmic reticulum (ER), where it is targeted for degradation. Curcumin induces the functional appearance of CFTR in the plasma membrane of airway cells, possibly through inhibition of the Ca^{2+}-ATPase of the ER, allowing for the transport of this channel to the plasma membrane

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[4,5,8]. Additionally, curcumin has recently been reported to directly stimulate the activity of the CFTR Cl-channel by prolonging channel open time and reducing channel closed time [9].

In addition to the CFTR chloride channel, curcumin has been shown to inhibit the inositol 1,4,5-triphosphate receptor calcium channel of porcine cerebellar microsomes [10]. Until now, curcumin has not been reported to modulate the activity of voltage-gated ion channels. In the present study, we found that curcumin potently and reversibly inhibits the voltage-gated, rapidly inactivating Kv1.4 K^+ channels expressed by bovine adrenal zona fasciculate cells [11].

Materials and methods

Tissue culture media, antibiotics, fibronectin, and fetal bovine sera (FBS) were obtained from Invitrogen (Carlsbad, CA). Coverslips were from Bellco (Vineland, NJ). Phosphate-buffered saline (PBS), enzymes, 1,2-bis-(2-aminophenoxy)ethane-*N*,*N*,*N′*,*N′′*-tetraacetic acid (BAPTA), cyclic AMP, and ATP were from Sigma (St. Louis). Curcumin was purchased from Biomol (Plymouth Meeting, PA).

Isolation and culture of AZF cells

Bovine adrenal glands were obtained from steers (age 2–3 years) at a local slaughterhouse. Isolated AZF cells were obtained and prepared as previously described [12]. After isolation, cells were either resuspended in DMEM/F12 (1:1) with 10% FBS, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and the antioxidants 1 μM tocopherol, 20 nM selenite, and 100 μM ascorbic acid (DMEM/F12+), and plated for immediate use or resuspended in FBS/5% DMSO, divided into 1 ml aliquots, and stored in liquid nitrogen for future use. For patch-clamp experiments, cells were plated in DMEM/F12+ in 35 mm dishes containing 9 mm^2 glass coverslips. Coverslips were treated with fibronectin (10 μg/ml) at 37 °C for 30 min and then rinsed with warm, sterile PBS immediately before adding cells. Cells were maintained at 37 °C in a humidified atmosphere of 95% air-5% CO_2.

Recording conditions and electronics

AZF cells were used for experiments 2–12 h after plating. Typically, cells with diameters <15 μm and capacitances of 8–15 pF were selected. Coverslips were transferred from 35 mm culture dishes to the recording chamber (volume, 1.5 ml) which was perfused by gravity at a rate of 3–5 ml/min. Patch electrodes with resistances of 1.0–2.0MΩ were fabricated from Corning 0100 glass (WPI, Sarasota, FL) using a Brown-Flaming model P-97 microelectrode puller (Sutter Instruments, Novato, CA). K^+ currents were recorded at room temperature (22–25 °C) following the procedure of Hamill et al. [13] with a List-EPC 7 patch-clamp amplifier (Axon Instruments, Burlingame, CA).

Pulse generation and data acquisition were done using a personal computer and PCLAMP software with TL-1 interface (Axon Instruments). Currents were digitized at 2–20 KHz after filtering with an eight-pole Bessel filter (Frequency Devices, Haverhill, MA). Linear leak and capacity currents were subtracted from current records using scaled hyperpolarizing steps of one-third to one-fourth amplitude. Data were analyzed and plotted using PCLAMP 9 (Clampfit) and SigmaPlot (ver 8.0).

Recording solutions

For recording whole-cell K^+ currents, the standard pipette solution consisted of (in mM): 120 KCl, $2MgCl_2$, 1 $CaCl_2$, 10 Hepes, 11 BAPTA, 0.2 GTP, and 2 MgATP with pH titrated to 7.2 using KOH. With this composition, free $[Ca^{2+}]$ was determined to be 2.3×10^{-8} M using the "Bound and Determined" program [14]. The development of the noninactivating bTREK K^+

current that is present in these cells was completely eliminated by including 100 μM cAMP in the pipette solution. cAMP selectively inhibits the bTREK K^+ current and does not alter Kv1.4 [15]. Pipette solutions were filtered through 0.22 μ cellulose acetate filters. The external solution consisted of (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 Hepes, and 5 glucose, with pH adjusted to 7.3 using NaOH.

Results

Bovine AZF cells express two distinctive K^+ currents. These include a voltage-gated, rapidly inactivating Kv1.4 K^+ current and a background, two-pore domain bTREK-1 K^+ current [16, 17]. Kv1.4 can be isolated in whole-cell recordings by including 100 μM cAMP in the recording pipette to selectively block the bTREK-1 current [11].

The Kv1.4 K^+ current in AZF cells was potently blocked by curcumin with an IC_{50} of $4.4 \times 10^{-6} \pm 0.13$ μM and a Hill coefficient of 2.32 ± 0.24 (Fig. 1A and B). At a concentration of 10 μM, inhibition by curcumin reached a steady-state within 10 min and was slowly reversible upon washing (Fig. 1A).

Some antagonists preferentially bind to and block channels that are in the conformations corresponding to open or inactivated, rather than the closed state. Block by these agents is often use-dependent and enhanced by repeated depolarization [18–20]. Inhibition of Kv1.4 by curcumin displayed limited use-dependence. In the experiment illustrated in Fig. 2, K^+ currents were recorded in standard saline before superfusing curcumin (20 μM) with (●) or without (○) a 10 min stimulation-free period. With uninterrupted stimulation, Kv1.4 K^+ current was inhibited nearly completely after a 10 min exposure to curcumin (Fig. 2, right panel). By comparison, when superfusion of curcumin was accompanied by a 10 min pulse-free period, inhibition had reached maximum value of 74.8% upon resumption of depolarizing steps (Fig. 2, trace 2). When voltage steps were applied for an additional 5 min, inhibition reached a steady-state value of 97.6%. Overall, in a total of four experiments, curcumin inhibited Kv1.4 by 68.4 ± 6.5% in the absence of stimulation, while inhibition increased to 96.3 ± 3.9% (n = 4) when voltage steps were applied at 30 s intervals for 5 min.

Inhibition of Kv1.4 by curcumin could occur through a direct occlusion of the pore, or by an allosteric mechanism whereby preferential binding of this agent to the inactivated state of the channel produces a hyperpolarizing shift in the steady-state availability such that channels inactivate at more negative potentials [19,20]. The voltage-dependent steady-state inactivation of the Kv1.4 K^+ currents was assessed in the absence and presence of curcumin by applying 10 s conditioning pulses to various potentials between −80 and 0 mV, followed by activating steps to +20 mV. Normalized K^+ currents were averaged and the mean values were fit with a smooth curve according to the Boltzmann relationship $I/I_{max} = 1/[1+\exp(\upsilon - \upsilon_{1/2}/K)]$ where I_{max} is the current activated from a holding potential of −80 mV, $\upsilon_{1/2}$ is the voltage where 1/2 of the channels are in the open configuration, and K is the slope factor. Curcumin failed to significantly shift the voltage-dependence of Kv1.4 inactivation. In control saline, Kv1.4 inactivated with a $\upsilon_{1/2}$ of −51.7 ± 1.5 mV (n = 6), compared to −49.8 ± 2.7 mV (n = 3) in the presence of 5 μM curcumin.

Discussion

The findings of this study identify curcumin as one of the most potent organic antagonists of A-type voltage-gated K^+ currents yet described. Kv1.4 K^+ channels are widely distributed in excitable cells of mammalian tissues, including the brain and heart [21–23]. At the cellular level in neurons, rapidly inactivating K^+ channels function pivotally in regulating action potential waveform and firing frequency [18,24]. At the subcellular level in neurons, Kv1.4

K^+ channels are located at presynaptic nerve terminals where they may function in the regulation of transmitter release [25,26]. Consequently, the pharmacology of rapidly inactivating K^+ channels has been a subject of interest.

A summary of the pharmacology of Kv1.4 K^+ channels is provided in Table 1. Among the classic antagonists of voltage-gated K^+ channels, curcumin is more than 25 and 1000 times as potent as 4-aminopyridine and TEA, respectively, as inhibitors of these channels. Curcumin is also significantly more potent than each of the organic antagonists identified as Kv1.4 blockers to date. The inhibition curve for curcumin was steep, with a Hill coefficient of 2.321, indicating that multiple curcumin molecules interact with a single Kv1.4 channel.

Block of Kv1.4 by curcumin exhibited partial use-dependence and did not shift the voltage-dependence of steady-state inactivation. These results suggest that curcumin may bind with slightly higher affinity to open, rather than inactivated, K^+ channels and thus its potency increases under conditions of repeated depolarization where Kv1.4 channels spend a larger fraction of time in the open conformation [19].

Curcumin is consumed in large quantities in the diet of certain individuals, yet side effects in humans have rarely been reported. In a phase 1 clinical trial using curcumin as a chemoprotective agent for patients with pre-malignant lesions, curcumin was not toxic to humans in amounts up to 8000 mg/day [27]. However, peak serum levels in these patients were only 1.77 μM, a value several fold lower than our measured IC_{50} for inhibition of Kv1.4 K^+ current. It is conceivable that, if curcumin were consumed or administered in even higher amounts as a therapeutic or prophylactic agent, serum concentrations could be reached where block of Kv1.4 and perhaps other ion channels could occur. In this regard, the potency of curcumin as an inhibitor of Kv1.4 is similar to that reported for stimulation of the CFTR chloride channel, and for inhibition of the IP_3 receptor Ca^{2+} channel [9,10].

It is not known if any of the many therapeutic actions attributed to curcumin could occur through interaction with voltage-gated K^+ channels such as Kv1.4. However, K^+ channels including Kv1.4 regulate the proliferation of various cells, including oligodendrocyte progenitors and vascular smooth muscle cells, and pituitary adenomas [8,28–30]. Block of these K^+ channels could contribute to the anti-neoplastic actions of curcumin.

Acknowledgments

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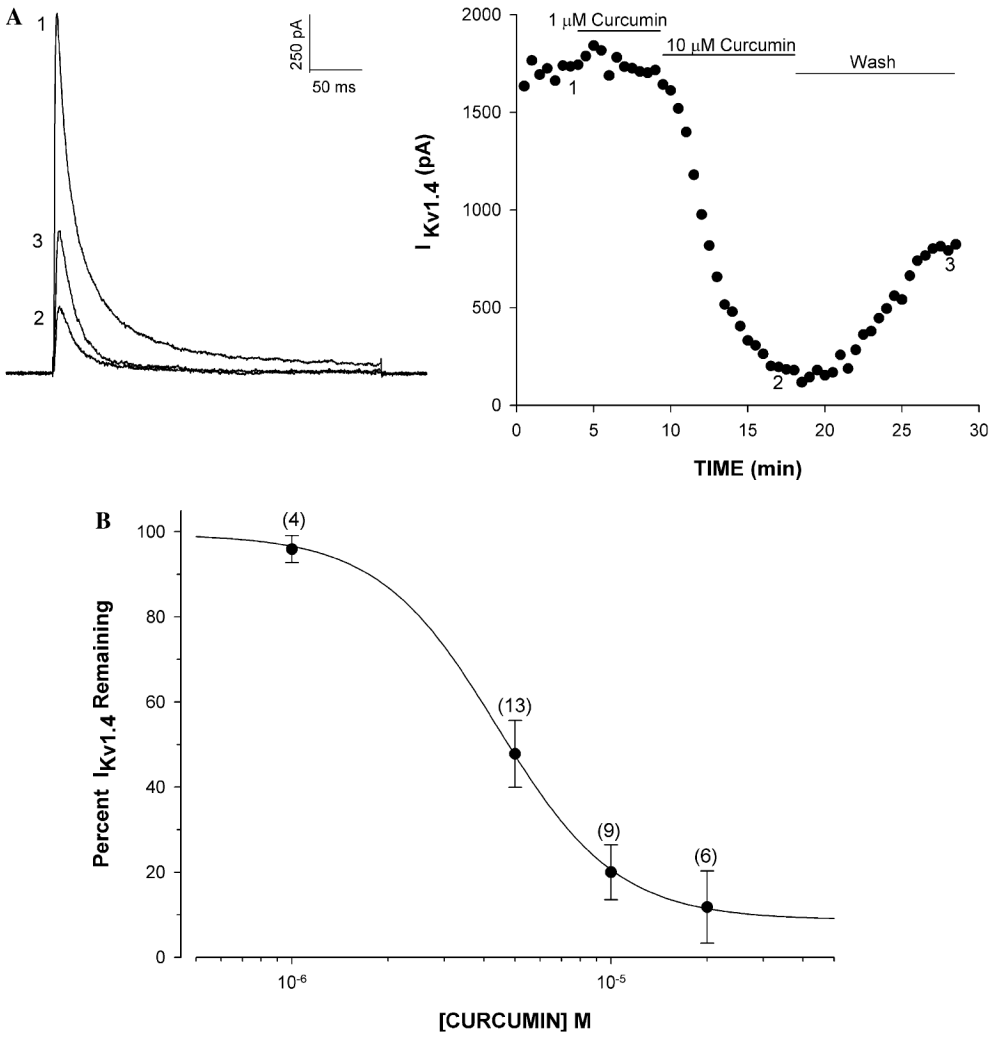

Fig. 1.

Concentration-dependent inhibition of Kv1.4 K^+ current by curcumin in bovine AZF cells. Kv1.4 K^+ current was activated from a holding potential of −80 mV by voltage steps to +20 mV, applied at 30 s intervals. After recording currents in standard saline, cells were superfused with curcumin at various concentrations. (A) K^+ current traces and corresponding plot of Kv1.4 peak amplitude against time for cell superfused with curcumin at the indicated concentrations. Numbers on traces correspond to currents recorded at times indicated on graph at right. (B) Inhibition curve for curcumin constructed from experiments as in (A). Percentage of unblocked current is plotted against curcumin concentration. Data are fit with the equation: $I/I_{max} \times 100 = 1/1 + (B/IC_{50})^x$ where B is curcumin concentration, IC_{50} is the concentration that reduces Kv1.4 by 50%, and x is the Hill slope.

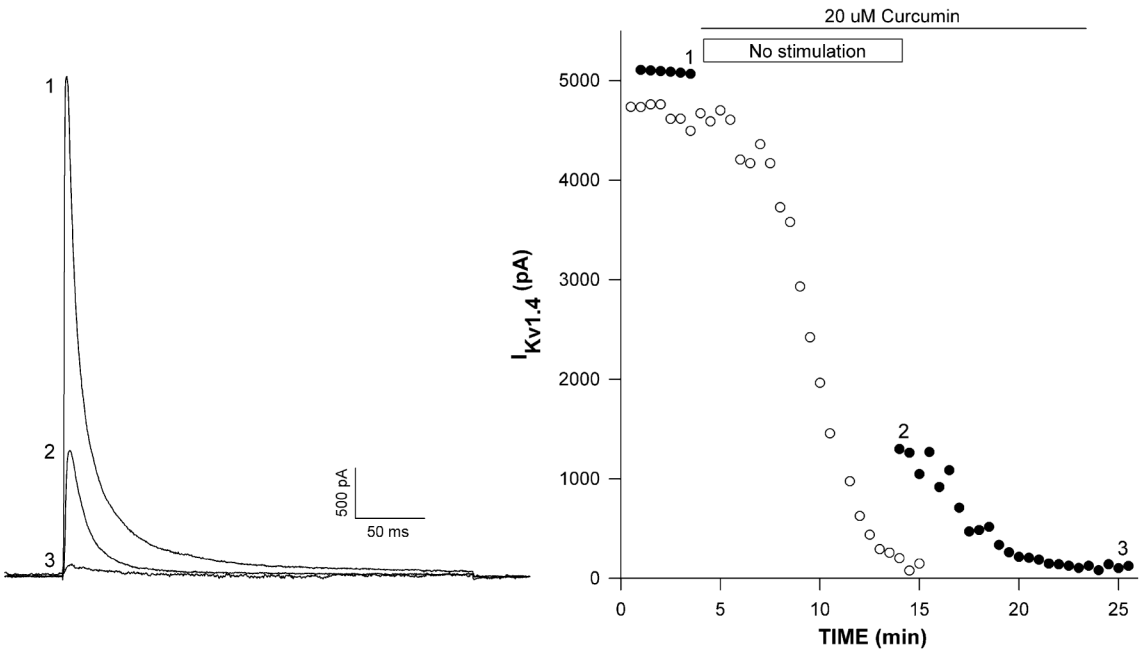

Fig. 2.

Use-dependent block of Kv1.4 K^+ current by curcumin. Kv1.4 K^+ currents were activated after recording currents at 30 s intervals from a holding potential of −80 mV by voltage steps to +20 mV. After recording currents in standard saline, cells were superfused with 20 μM curcumin with or without a 10 min pulse-free period. Kv1.4 K^+ current traces (left panel) and corresponding plot (right panel) of peak amplitudes against time (closed circles) for a cell superfused with curcumin for 10 min with no stimulation. Numbers on traces correspond to currents recorded at indicated times on graph at right. Open circles on plot show time-dependent inhibition by curcumin with uninterrupted stimulation.

Table 1

Pharmacology of Kv1.4 K^+ channels

Antagonist	IC_{50} (μM)	Reference
Curcumin	4.4	[31]
TEA	>10,000	[31]
4-AP	124.2	[31]
Quinidine	74.6	[31]
Fluoxetine	33.1	[32]
Flecainide	706	[33]
Clofilium	1071	[34]
Disopyrimide	>1000	[34]
Verapamil	707.9	[34]
Bertosamil	323.6	[34]
La^{3+}	33.7	[35]
Ni^{2+}	467	[35]