Capsaicin Inhibits Jurkat T-Cell Activation by Blocking Calcium Entry Current I_{CRAC}

BRUCE S. FISCHER, DANMEI QIN, KAMI KIM, and THOMAS V. MCDONALD

Departments of Medicine (B.S.F., K.K., T.V.M.), Molecular Pharmacology (D.Q., T.V.M.), and Microbiology and Immunology (K.K.), Albert Einstein College of Medicine, Bronx, New York

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ABSTRACT

Capacitative calcium entry (CCE) through stores-operated Ca^{2+} channels is an absolute requirement for normal activation of T lymphocytes. Organic blockers/inhibitors of the channel(s) that carry the inward Ca^{2+} current (I_{CRAC}) responsible for CCE are few. Here we show that capsaicin, the pungent ingredient of hot chili pepper, blocks receptor-stimulated Ca^{2+} entry in Jurkat T cells. Indo-1 measurements of intracellular calcium show that capsaicin blocks CCE without affecting release of inositol-1,4,5-trisphosphate-sensitive internal Ca^{2+} stores with an IC_{50} of 32 μM. Block of Ca^{2+} entry by capsaicin is identical whether CCE is evoked by T-cell receptor (TCR) stimulation, heterologous muscarinic M1 receptor stimulation, or via thapsigargin depletion of internal Ca^{2+} stores. Patch-clamp experiments show that capsaicin rapidly and reversibly blocks I_{CRAC} with an identical dose response as seen with indo-1 measurements.

Capsaicin, the piquant component in hot chili peppers, is a member of the vanilloid family. The biological activity of capsaicin has been the subject of extensive research (Szallasi and Blumberg, 1999). The identified pharmacological targets of capsaicin are primarily sensory nerve fibers where high nanomolar concentrations evoke relatively nonselective, calcium-permeable channel openings. One definitive molecular target of capsaicin is the vanilloid receptor(s) (VR) ion channel(s) that is activated by capsaicin. VR1, the first to be cloned, encodes an ion channel with sequence similarities to the transient receptor potential-related family of channels (Caterina et al., 1997). There is evidence that additional receptors and pharmacological targets for capsaicin exist (Petersen et al., 1996; Biro et al., 1998; Liu et al., 1998). A variety of other ion channel types have been shown to be sensitive to block by capsaicin at micromolar concentrations (Szallasi and Blumberg, 1999). Choi and Kim (1999) demonstrated, in an intriguing report, that micromolar concentrations of capsaicin inhibited capacitative Ca^{2+} entry (CCE) in the neuroendocrine cell line PC12. They showed, with 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methylphenoxy)-ethane-N,N′,N′-tetraacetic acid [Ca^{2+}], measurements, that the inhibition of CCE was not due to alteration of inositol-1,4,5-trisphosphate (InsP3) release of internal Ca^{2+} stores but, specifically, reduction of Ca^{2+} entry. In the same report the authors also provided evidence that capsaicin had a similar effect on CCE in Jurkat T cells.

We report here a detailed examination of capsaicin inhibition of CCE in Jurkat T cells. Jurkat cells are a human lymphoblastic cell line that has been used extensively to study early signal transduction events in T-lymphocyte activation through the T-cell receptor (TCR) (Binstadt et al., 2000). Our results confirm that capsaicin inhibits CCE in Jurkat cells in a manner similar to that reported for PC12 cells. Furthermore, we identify the pharmacological target as the calcium-release-activated calcium current I_{CRAC}. We also show that capsaicin blocks the main voltage-gated K^{+} current.

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ABBREVIATIONS: VR, vanilloid receptor; CCE, capacitative calcium entry; [Ca^{2+}], intracellular calcium concentration; InsP3, inositol-1,4,5-trisphosphate; TCR, T-cell receptor; I_{CRAC}, calcium-release-activated calcium current; IL-2, interleukin-2; ELISA, enzyme-linked immunosorbent assay; CPZ, capsazepine; RTx, resiniferatoxin; PMA, phorbol myristate acetate; PHA, phytohemagglutinin; CCh, carbachol; CaT1, calcium transport protein; ECaC, epithelial calcium channel.
dent produced by Kv1.3, an effect that may contribute to inhibition of CCE through reduction of the electrochemical gradient for Ca\textsuperscript{2+} entry. Direct block of I\textsubscript{CRAC} appears to play the largest role in inhibiting CCE. Inhibition of TCR-dependent production of IL-2 by capsaicin parallels CCE inhibition, suggesting that capsaicin block of I\textsubscript{CRAC} is biologically significant.

Materials and Methods

Cell Culture. The human leukemic T-cell line Jurkat (E6; American Type Culture Collection, Manassas, VA) and the Jurkat derivative JHM1.2.2 that stably expresses the human muscarinic receptor M1 (Goldsmith et al., 1989; McDonald et al., 1993) were maintained in suspension culture in RPMI 1640 medium (Cellgro, Herndon, VA) supplemented with 10% (v/v) heat-inactivated fetal calf serum (In vivogen, Carlsbad, CA) and 1% (v/v) penicillin/streptomycin (10,000 IU/ml/10,000 mg/ml; Cellgro). The cultures were kept at 37°C in a humidified 95% air, 5% CO\textsubscript{2} atmosphere. JHM1 cell media contained H-292 at 1 mg/ml. The cultures were prepared with varying concentrations of inhibitors (capsaicin, capsaazepine (CPZ), resiniferatoxin (RTX), and mepiquat) and either TCR-dependent stimulants (80 nM PMA and 10 μg/ml PHA) or TCR-independent stimulants (80 nM PMA and 1 μM ionomycin). Cell suspension (150 μl) was added to each well and incubated for 24 h at 37°C. Culture supernatants were harvested and diluted 1:200 to bring the IL-2 concentrations within the linear range of the ELISA. ELISA detection was performed as suggested by the manufacturer (BD Pharmingen) and colorimetric readings determined on automatic plate reader (Bio-Rad, Richmond, CA).

Electrophysiology. The parent cell line Jurkat E6 was used for patch-clamp electrophysiological studies. Extracellular solution was 170 mM Tris-Cl, 5 mM KCl, 1 mM MgCl\textsubscript{2}, 2.5 mM CaCl\textsubscript{2}, 5 mM glucose, 10 mM HEPES, pH 7.4. Intracellular pipette solution was 80 mM Cs-aspartate, 8 mM CsCl, 2 mM MgCl\textsubscript{2}, 20 mM Cs-EGTA, 20 mM HEPES, pH 7.2, with osmolality 300 to 330 mosm when measuring Ca\textsuperscript{2+} currents. For measuring K’ currents the pipette solution contained 120 mM KCl, 0.5 mM CaCl\textsubscript{2}, 5 mM EGTA, 4 mM ATP-K, 2 mM MgCl\textsubscript{2}, 20 mM HEPES, pH 7.2, with osmolality 270 to 290 mosmol. All experiments and solutions were at room temperature (−21°C). JHM1 cells were placed in a chamber connected to a perfusion system that exchanged the extracellular solution within 10 to 15 s. The perfusion chamber was mounted on the stage of an inverted microscope (Nikon, Tokyo, Japan).

The whole cell patch-clamp technique was used to record the Ca\textsuperscript{2+} and K’ currents. An Axopatch 200B patch-clamp amplifier and a Digidata 1320A 16-bit AD/DA digitizer (Axon Instruments, Foster City, CA) were controlled by a Pentium-based PC running pClamp 8 acquisition and analysis software (Axon Instruments). Voltage-clamp protocols used a holding potential of −40 mV and successive steps from −120 to 60 mV (in 20-mV increments) for 350 ms were used to generate current-voltage relationships. Voltage-clamp ramps were generated from a holding potential of −40 mV and ramped between −100 and 50 mV over 300 ms. Current signals were analog filtered at 2 kHz, digitally sampled at 5 to 6 kHz, and recorded to optical disc for storage and off-line analysis. Data were analyzed with Clampfit software version 8.0.3.128 (Axon Instruments) and with Origin for Windows version 6.0 (MicroCal Software).

Measurement of IL-2 Production. Quantification of IL-2 production from Jurkat T lymphocytes was performed using a commercial ELISA system (BD Pharmingen, San Diego, CA). Cells were grown to log phase, harvested by centrifugation, counted with a hemocytometer, and resuspended in fresh media at 10⁶ cells/ml. Co-stimulated cultures were prepared with varying concentrations of inhibitors (capsaicin, capsaazepine (CPZ), resiniferatoxin (RTX), and mepiquat) and either TCR-dependent stimulants (80 nM PMA and 10 μg/ml PHA) or TCR-independent stimulants (80 nM PMA and 1 μM ionomycin). Cell suspension (150 μl) was added to each well and incubated for 24 h at 37°C. Culture supernatants were harvested and diluted 1:200 to bring the IL-2 concentrations within the linear range of the ELISA. IL-2 ELISA detection was performed as suggested by the manufacturer (BD Pharmingen) and colorimetric readings determined on automatic plate reader (Bio-Rad, Richmond, CA).

Results

We examined receptor-activated Ca\textsuperscript{2+} signals by using indo-1-loaded Jurkat lymphocytes from both the parent clone (clone E6) and clone JHM1.1 that had been stably transfected with the human muscarinic receptor type 1 (Goldsmith et al., 1989). As previously demonstrated (McDonald et al., 1993), addition of carbachol (CCh, 250 μM) to JHM1.1 cells evoked a rapid rise in [Ca\textsuperscript{2+}]i to a concentration of several micromolar, which then declined to 1.2 μM (Fig. 1, A and B). The initial rise in [Ca\textsuperscript{2+}]i is produced by the release of internal Ca\textsuperscript{2+} stores from InsP3-sensitive pools that are limited and thus, result in a transient signal. When external calcium is absent, isolated release of internal Ca\textsuperscript{2+} stores can be seen as the brief signal after CCh is applied (Fig. 1C). Upon addition of millimolar external Ca\textsuperscript{2+} a brisk elevation of [Ca\textsuperscript{2+}]i is produced that represents CCE through surface Ca\textsuperscript{2+}-permeable channels (Fig. 1C). Capsaicin inhibited CCE in Jurkat T lymphocytes as measured by indo-1. When capsaicin was added prior to stimulation of cells, the initial InsP3-dependent rise in [Ca\textsuperscript{2+}]i, remained largely unchanged but the ensuing plateau of [Ca\textsuperscript{2+}]i, was substantially decreased (Fig. 1A). When capsaicin was applied after CCh stimulation the sustained [Ca\textsuperscript{2+}]i plateau was quickly abolished (Fig. 1B). In experiments where release of internal Ca\textsuperscript{2+} stores was separated from Ca\textsuperscript{2+} entry (Fig. 1C), pretreatment with capsaicin had no effect on InsP3-dependent Ca\textsuperscript{2+} release at concentrations that blocked CCE. The block of CCE by capsaicin was dose-dependent with an IC\textsubscript{50} of 32 μM (Fig. 1D), a value similar to that obtained for PC12 cells (Choi and Kim, 1999). The muscarinic M1 receptor is a G protein (G\textsubscript{q})-coupled receptor whose stimulation results in rapid activation of phospholipase C that hydrolyzes phosphatidyl-inositide-4,5-
bisphosphate, generating InsP3 and diacylglycerol. The usual mechanism for T-lymphocyte activation is through the TCR that begins a cascade of tyrosine kinase activity, leading to phosphorylation of phospholipase C and generation of InsP3.

To determine whether capsaicin effects on CCE were independent of the receptor or signals leading up to generation of InsP3 we examined \([\text{Ca}^{2+}]_i\) signals with indo-1 in the parent cell line Jurkat E6, stimulated via the TCR with either phytohemagglutinin A or an anti-CD3 antibody (UCHT1). In both cases capsaicin produced the same block of CCE as observed in CCh-stimulated cells (Fig. 2, A and B). When we bypassed InsP3 signaling in Jurkat cells by depletion of the internal \([\text{Ca}^{2+}]_i\) stores with the smooth endoplasmic reticulum Ca-ATPase inhibitor thapsigargin, capsaicin continued to inhibit the CCE (Fig. 2C). Taken together, results illustrated in Figs. 1 and 2 show that capsaicin inhibits Jurkat CCE without altering receptor signaling, generation of InsP3, or release of internal \([\text{Ca}^{2+}]_i\) stores. The possible mechanisms include direct block of the \([\text{Ca}^{2+}]_i\) influx channels, disruption of the electrochemical driving force for \([\text{Ca}^{2+}]_i\) entry, or inhibition of the signal between release of internal stores and opening of surface channels.

Many analogs of capsaicin with varying activities have been discovered or synthesized (Walpole et al., 1993). We examined the effects of one naturally occurring analog, RTx, and one synthetic analog, CPZ, on Jurkat CCE. RTx is an ultrapotent activator of native and cloned VR (Szallasi and Blumberg, 1989; Caterina et al., 1997). With RTx we observed inhibition of stores-operated \([\text{Ca}^{2+}]_i\) entry in Jurkat cells moderately more potently than capsaicin (IC\(_{50}\) 1 \(\mu M\) (Fig. 3, A and B). CPZ was synthesized as a competitive inhibitor of capsaicin’s binding and activation of native VR (Dickenson and Dray, 1991). In Jurkat cells CPZ also inhibits stores-operated \([\text{Ca}^{2+}]_i\) entry with an IC\(_{50}\) = 6 \(\mu M\) (Fig. 3, C and D).

In T lymphocytes and many other cell types depletion of internal stores of \([\text{Ca}^{2+}]_i\) with InsP3 stimulates the opening of plasma membrane channels that are selective for \([\text{Ca}^{2+}]_i\) permeation and carry a current, \(I_{\text{CRAC}}\) (Lewis and Cahalan, 1989; Hoth and Penner, 1992; McDonald et al., 1993; Zweifach and Lewis, 1993). To determine whether \(I_{\text{CRAC}}\) was the...
Fig. 2. Capsaicin blocks stores-operated Ca\(^{2+}\) entry in Jurkat cells. Stores-operated Ca\(^{2+}\) entry in Jurkat cells is blocked by capsaicin regardless of the method of stimulation. A, Jurkat cells stimulated with PHA (10 μg/ml) followed by capsaicin (100 μM). B, Jurkat cells stimulated by α-CD3 monoclonal antibody (UCHT1) and anti-mouse IgG cross-linking antibody followed by capsaicin (100 μM). C, Jurkat cells stimulated by thapsigargin (0.5 μM) followed by capsaicin (100 μM).

Fig. 3. Capsaicin analogs block stores-operated Ca\(^{2+}\) entry in Jurkat cells. [Ca\(^{2+}\)]\(_i\) measurements in indo-1-loaded JHM1 2.2 cells. A, cells stimulated by CCh (250 μM) followed by capsaicin (100 μM). B, summary dose response for RTx inhibition of Ca\(^{2+}\) plateau entry in Jurkat cells, IC\(_{50}\) = 1 μM. C, cells stimulated by PHA (10 μg/ml) followed by capsazepine (10 μM). D, summary dose response for capsazepine inhibition of Ca\(^{2+}\) plateau entry in Jurkat cells, IC\(_{50}\) = 6 μM.
target of the capsaicin effects we applied capsaicin to Jurkat cells during whole-cell voltage-clamp experiments. After establishing maximal inward Ca\(^{2+}\) currents through Ca\(^{2+}\) stores depletion by means of thapsigargin and internal Ca\(^{2+}\) chelation, repetitive ramp pulses were applied and external solutions were changed to those containing various concentrations of capsaicin. Capsaicin produced sustained block of I\(_{\text{CRAC}}\) that was dose-dependent with an IC\(_{50}\) = 30 \(\mu\)M (Fig. 4, A and C). There was no evidence of voltage dependence of channel block. The portion of current blocked was the same at all voltages at a given concentration and there was no there any difference in block with or without repetitive depolarizing pulses (Fig. 4B). The onset of block was rapid, within 5 s from the start of external solution change. The block by capsaicin was rapidly reversible with washout of the external solution with a time course that mirrored the onset of block (Fig. 4D).

Chandy and colleagues previously reported that capsaicin blocks a number of cloned voltage-gated K\(^+\) channels, including Kv1.3, the primary K\(^+\) current expressed in T lymphocytes (Grissmer et al., 1994). To further examine the specificity of capsaicin for I\(_{\text{CRAC}}\) in Jurkat cells, we performed whole cell voltage clamp on Jurkat cells by using internal and external solutions and voltage protocols to optimize measurements of voltage-gated K\(^+\) currents. When capsaicin was applied to cells, the voltage-gated K\(^+\) current consistent with Kv1.3 was rapidly blocked in a nonvoltage-dependent manner with an IC\(_{50}\) = 18 \(\mu\)M (Fig. 5). To determine whether block of I\(_{\text{CRAC}}\) and Kv1.3 were coupled we examined the effects a potent peptide inhibitor of Kv1.3, margatoxin (Garcia-Calvo et al., 1993). During voltage-clamp studies margatoxin completely blocked all voltage-gated K\(^+\) current in Jurkat cells at concentrations between 200 pM and 1 nM (Fig. 6). When concentrations up to 10 nM were applied during measurements of I\(_{\text{CRAC}}\) however, there was no effect (Fig. 6B).

To determine whether block of I\(_{\text{CRAC}}\) by capsaicin could result in functional immunosuppressive activity TCR activation was measured in the presence of capsaicin (Fig. 7). Jurkat cells were stimulated by a TCR-dependent regimen (10 \(\mu\)g/ml PHA plus 80 nM PMA) for 24 h in the presence of varying concentrations of capsaicin. TCR-independent stimulation was performed with 1 \(\mu\)M ionomycin plus 80 nM PMA. Both TCR-dependent and -independent production of IL-2 was suppressed by capsaicin in a dose-dependent manner. TCR-dependent IL-2 production was more sensitive to suppression by capsaicin with an IC\(_{50}\) = 18 \(\mu\)M. TCR-independent IL-2 production was inhibited by capsaicin with an IC\(_{50}\) = 75 \(\mu\)M. The TCR-dependent sensitivity to suppression by capsaicin more closely corresponds with the dose-response seen for I\(_{\text{CRAC}}\) (IC\(_{50}\) = 30 \(\mu\)M). The capsaicin analogs resini-
eratoxin and capsazepine both inhibited IL-2 production, however, margatoxin did not.

**Discussion**

Because of the importance of CCE in T-lymphocyte activation, we sought to define the mechanism(s) by which capsaicin treatment affects Ca$^{2+}$ signaling in T lymphocytes and to determine the consequences of capsaicin on lymphocyte function. Several mechanisms for drug inhibition of CCE are possible, including inhibition of receptor-mediated signaling, inhibition of release of Ca$^{2+}$ stores, decreased driving force (electrochemical gradient) for Ca$^{2+}$ entry, disruption of the signal between Ca$^{2+}$ stores release and Ca$^{2+}$ entry, and direct block of the Ca$^{2+}$ entry channels that produce I_{CRAC}. Capsaicin blocked CCE generated either through TCR stimulation or via ligation of a heterologous muscarinic M1 receptor and also blocked the CCE induced by thapsigargin, indicating that the capsaicin effect is not due to alteration of receptor-mediated signaling. Previous reports demonstrating that capsaicin has blocking effects on other ion channels suggested to us that either I_{CRAC} or disruption of the electrochemical gradient was the target for capsaicin-mediated effects in lymphocytes.

Our results from patch-clamp current measurements show that capsaicin (and the analogs capsazepine and RTx) rapidly and reversibly block I_{CRAC} in a dose-dependent manner, indicating a direct effect on channel proteins. These experiments were performed under voltage clamp, negating any effect capsaicin may have on membrane potential and electrochemical driving force. The dose response of the current block is sufficient to account for CCE inhibition observed in the indo-1 studies. That the block was complete within several seconds of administration and was rapidly reversible supports a direct channel-blocking mechanism. The rapid reversibility was rather surprising given the lipophilic nature of these drugs but argues for a direct drug-channel interaction rather than a nonspecific alteration in plasma membrane. When the drug SKF96365 was used in similar studies, the onset of block was 30 to 60 s, suggesting an indirect inhibition mechanism (Chung et al., 1994).

The predominant K$^+$ current expressed in quiescent T lymphocytes is the voltage-activated current carried by the channel Kv1.3 (Lee et al., 1992). Kv1.3 is thought to be involved in maintaining the membrane potential at negative values near the equilibrium potential for K$^+$. As such, drugs that alter K$^+$ channel activity could affect the driving force for Ca$^{2+}$ entry. Capsaicin blocked the voltage-gated K$^+$ current with a dose response similar to its block of I_{CRAC}, consistent with previously reported effects on voltage-gated K$^+$ channels (Grissmer et al., 1994). Although capsaicin block of Kv1.3 may reduce the electrochemical forces favoring Ca$^{2+}$ entry, it does not appear to be a significant factor in altering CCE in Jurkat cells in our system. Margatoxin, the specific peptide blocker of Kv1.3, failed to reduce CCE when [Ca$^{2+}$], was measured with indo-1 and supports our conclusion that

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**Fig. 5.** Lymphocyte voltage-activated K$^+$ current is blocked by capsaicin. Whole cell voltage-clamp recordings of the voltage-gated K$^+$ current in Jurkat cells characteristic of Kv1.3. A, families of current traces from one representative cell superimposed during voltage steps to between −60 and 60 mV. Traces on the left show outward K$^+$ currents at baseline. Traces in the middle show block of K$^+$ currents after application of capsaicin (100 μM). Traces on the right show return of K$^+$ currents after washout of capsaicin. B, summary dose response for capsaicin block of voltage-gated K$^+$ currents in Jurkat cells, IC$_{50}$ = 18 μM.
capsaicin inhibits Ca\(^{2+}\) entry primarily by direct block of I\(_{\text{CRAC}}\). Moreover, when margatoxin was applied to lymphocytes at concentrations 50- to 100-fold higher than the IC\(_{50}\) for Kv1.3 we observed no effect on I\(_{\text{CRAC}}\) in voltage-clamp studies. Although some investigators have demonstrated that K\(^{+}\) channel blockers have immunosuppressive effects, others have reported a lack of effect (Kerschbaum et al., 1997). This may be due to timing or concentration of administered drugs, or it may reflect the change in K\(^{+}\) channel gene expression pattern that occurs after TCR stimulation (Ganshani et al., 2000).

Our observation that low micromolar concentrations of capsaicin block two unrelated channels could be interpreted as a nonspecific effect on the lipid membrane by this hydrophobic compound. Meddings et al. (1991) reported on capsaicin-induced changes in membrane fluidity in a variety of cell types. These effects, however, were seen at capsaicin concentrations exceeding 150 \(\mu\)M, well above the IC\(_{50}\) values we observed. Moreover, they found that the membrane effects varied with cell type and that lymphocytes were fairly resistant to the nonspecific lipid effects of capsaicin. Arguing against a nonspecific effect from capsaicin preferentially partitioning into the lipid membrane is our finding that the I\(_{\text{CRAC}}\) block was reversible during washout within the time frame of bath chamber exchange (Fig. 4D). If capsaicin’s effects were simply due to its high partitioning into the lipid bilayer membrane with subsequent fluidity changes the reversibility would be expected to be much slower. We did observe nonspecific effects of capsaicin when we used concentrations greater than 250 \(\mu\)M, which resulted in a slowly developing and irreversible equilibration of internal and external Ca\(^{2+}\) concentrations that was accompanied by a large nonselective increase in membrane conductance (data not shown).

Biological consequences of capsaicin-mediated block of CCE are seen in T-lymphocyte activation. One of the earliest signaling events in normal activation of T lymphocytes through the TCR is an InsP3-mediated elevation of [Ca\(^{2+}\)]\(_i\), that is comprised of a release of internal stores followed by CCE (Tsien et al., 1982). This elevation of [Ca\(^{2+}\)]\(_i\) may be oscillatory (Lewis and Cahalan, 1989; Dolmetsch and Lewis, 1994) and must be sustained for 30 to 45 min for commitment of the lymphocyte down an activated pathway as heralded by new transcription of the cytokine IL-2 (Crabtree, 1999).
Capsaicin analogs capsazepine and RTx also inhibited TCR-dependent IL-2 production at concentrations that blocked CCE. Margatoxin, however, failed to alter TCR-dependent IL-2 production, supporting our conclusion that selective block of Kv1.3 failed to significantly reduce CCE.

The mechanism for this symptomatic relief of pain by capsaicin has been ascribed to selective effects on neurons that are responsible for sensing painful stimuli (Szallasi and Blumberg, 1999). Capsaicin stimulates the opening of VR1 channels and results in prolonged elevation of [Ca\(^{2+}\)], and ensuing apopto-sis of the pain fibers and decreased local secretion of substance P (Szallasi and Blumberg, 1999). Our results suggest the existence of additional, local anti-inflammatory effect of capsaicin due to effects upon T-lymphocyte ion channels. Supportive of our findings are the numerous reports of capsaicin’s immunomodulatory effects from both in vitro and in vivo studies (Nilsson et al., 1991; Biro et al., 1998; Lai et al., 1998; Szallasi and Blumberg, 1999).

The channel protein(s) responsible for I\(_{\text{CRAC}}\) in lymphocytes have not yet been definitively identified. Caterina et al. (1997) used expression cloning of a capsaicin-responsive ion channel to identify the first vanilloid receptor VR1. VR1 is expressed, to a lesser degree, in a variety of tissues other than sensory neurons (Hayes et al., 2000; Mezey et al., 2000; Schumacher et al., 2000). The effects of capsaicin described in this report, however, are unlikely to be due to VR1 expression in lymphocytes because we observe a block of Ca\(^{2+}\) flux, whereas capsaicin would stimulate flux through VR1 channels. Several reports have recently identified channel proteins (ECaC1 and CaT1) from proximal small intestine that exhibit biophysical properties closely resembling I\(_{\text{CRAC}}\) (Hoenderop et al., 1999; Peng et al., 1999). ECaC1 and CaT1 have sequence homology to the broader class of transient receptor potential-related channels that include VR1 and vanilloid receptor-like gene-1 (Caterina and Julius, 2001). The initial Northern analyses of ECaC1 and CaT1 did not show expression in lymphoid tissue (Hoenderop et al., 1999; Peng et al., 1999; Yue et al., 2001); however, a later report states that CaT1 was detected by reverse transcription-polymerase chain reaction from Jurkat cells (Yue et al., 2001). We have also obtained full-length vanilloid receptor-like gene-1 mRNA from Jurkat (data not shown). These data suggest that one or more of these channels may play a role in T-lymphocyte CCE. The capsaicin sensitivity of these channels expressed in a heterologous system may help to clarify the molecular components of lymphocyte I\(_{\text{CRAC}}\).

Many analogs of capsaicin have been discovered and synthesized that have varying agonist and antagonist activities on pain fiber vanilloid receptors. We show that one antagonist (capsazepine) and one ultrapotent analog (RTx) block of I\(_{\text{CRAC}}\) and CCE in Jurkat lymphocytes at lower concentrations than capsaicin. Research in animal models and humans will be needed to further explore and validate the anti-inflammatory properties of capsaicin. Further testing of other capsaicin analogs may lead to development of additional immunomodulatory drugs.

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**Fig. 7.** Capsaicin and its analogs suppress TCR-mediated IL-2 Production in Jurkat Cells. A, summary dose-response data of capsaicin block of IL-2 production in response to stimulation with a combination of 10 μg/ml PHA and 80 nM PMA (TCR-dependent, ○) or 1 μM ionomycin and 80 nM PMA (TCR-independent, □). IC\(_{50}\) = 15 μM for block of TCR-dependent IL-2 production. IC\(_{50}\) = 75 μM for block of TCR-independent IL-2 production. B, IL-2 production from Jurkat cells stimulated with PHA and PMA in the presence of capsaicin (100 μM), capsazepine (25 μM), resiferatoxin (20 μM), or margatoxin (10 nM).

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new production of IL-2 is achieved by calcium-calmodulin stimulation of calcineurin, which leads to dephosphorylation of the transcription factor NFAT (Crabtree, 1999). If [Ca\(^{2+}\)]\(_i\) declines too early after TCR stimulation a reverse translocation of NFAT out of the nucleus will occur (Shibasaki et al., 1996), thus aborting the immune response. Immunomodulatory drugs (cyclosporin, FK506) are used that target calcineurin and inhibit translocation of NFAT. Pharmacological agents that can alter lymphocyte CCE can modulate immune responsiveness and may be useful alternatives or adjuncts to established immunosuppression therapies (Chung et al., 1994). Our results show that capsaicin, at concentrations that block CCE, inhibits TCR-dependent production of IL-2 in Jurkat cells. The production of IL-2 in cells stimulated by calcium ionophore and phorbol ester required higher concentrations of capsaicin to inhibit. This may be explained by ionomycin’s ability to deplete internal stores of Ca\(^{2+}\), causing influx of external Ca\(^{2+}\) through I\(_{\text{CRAC}}\) as well as through the ionophore’s direct effect on the plasma membrane.
References


Address correspondence to: Thomas V. McDonald, Departments of Medicine and Molecular Pharmacology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461. E-mail: mcdonald@ae.com, yu.edu