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*J Immunol* 2000; 165:3992-3998; doi: 10.4049/jimmunol.165.7.3992
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Capsaicin Inhibits Platelet-Activating Factor-Induced Cytosolic Ca\(^{2+}\) Rise and Superoxide Production\(^1\)

Se-Young Choi,* Hyunjung Ha,† and Kyong-Tai Kim\(^2\)*

Platelet-activating factor (PAF)\(^3\) is one of several other important modulators of the inflammatory process. We studied the regulation of PAF activity by capsaicin in human promyelocytic leukemia HL-60 cells. Capsaicin inhibited PAF-induced superoxide production in a concentration-dependent manner. In addition to PAF, the FMLP- and extracellular ATP-induced superoxide productions were inhibited by capsaicin, whereas PMA-induced superoxide production was not affected. In the PAF-stimulated cytosolic Ca\(^{2+}\) increase, capsaicin inhibited in particular the sustained portion of the raised Ca\(^{2+}\) level without attenuation of the peak height. In the absence of extracellular Ca\(^{2+}\), the PAF-induced Ca\(^{2+}\) elevation was not inhibited by capsaicin because capsaicin only inhibited the Ca\(^{2+}\) influx from the extracellular space. In addition, capsaicin did not affect PAF-induced inositol 1,4,5-trisphosphate production, suggesting that phospholipase C activation by PAF is not affected by capsaicin. Store-operated Ca\(^{2+}\) entry (SOCE) induced by thapsigargin was inhibited by capsaicin in a concentration-dependent manner. This capsaicin effect was also observed on thapsigargin-induced Ba\(^{2+}\) and Mn\(^{2+}\) influx. Furthermore, capsaicin’s inhibitory effect on the thapsigargin-induced Ca\(^{2+}\) rise overlapped with that of SK&F96365, an inhibitor of SOCE. Both capsaicin and SK&F96365 also inhibited PAF-induced cytosolic superoxide generation in HL-60 cells differentially by all-trans-retinoic acid. Our data suggest that capsaicin exerts its anti-inflammatory effect by inhibiting SOCE elicited via PLC activation, which occurs upon PAF activation and results in the subsequent superoxide production. The Journal of Immunology, 2000, 165: 3992–3998.

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Received for publication September 20, 1999. Accepted for publication July 17, 2000.

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\(^3\)Abbreviations used in this paper: PAF, platelet-activating factor (1-O-alkyl-2-acyl-sn-glycero-3-phosphocholine); SOCE, store-operated Ca\(^{2+}\) entry; [Ca\(^{2+}\)]\(_i\), cytosolic calcium ion concentration; PKC, protein kinase C; PLC, phospholipase C; Ins\(_{1,4,5}\), inositol 1,4,5-trisphosphate; fura-2/AM, fura-2 penta-acetoxymethyl ester; DCFH-DA, 2',7'-dichlorofluorescein diacetate.
Materials and Methods

Materials

Capsaicin, PAF, ATP, fMLP, thapsigargin, SK&F96365, cytochrome c, and sulfinpyrazone were purchased from Sigma (St. Louis, MO). Fura-2-penta-acetoxyethyl ester (fura-2/AM) and 2',7'-dichlorofluorescin diacetate (DCFH-DA) were obtained from Molecular Probes (Eugene, OR). [3H]Norepinephrine and [3H]InsP3, were purchased from NEN (Boston, MA). RPMI 1640 and penicillin/streptomycin were obtained from Life Technologies (Grand Island, NY). Bovine calf serum and horse serum were obtained from HyClone (Logan, UT). 

Cell culture

HL-60 cells were grown in RPMI 1640 supplemented with 10% (v/v) heat-inactivated bovine calf serum, 5% (v/v) heat-inactivated horse serum, and 1% (v/v) penicillin/streptomycin. The culture medium was changed daily. All cells were cultured in a humidified atmosphere of 95% air and 5% CO2. We induced differentiation of the HL-60 cells by incubating them in 1 μM all-trans-retinoic acid for 5 days (26). We observed the morphology of the cells and monitored the fMLP-induced cytosolic calcium ion concentration ([Ca2+]i) rise, which is only detectable in differentiated HL-60, as indicators of differentiation into neutrophil-like cells. We counted viable cells by the trypan blue exclusion method.

Measurement of superoxide secretion

Superoxide generation was determined based on the change in absorbance of cytochrome c using a previously published procedure with slight modification (27). Briefly, 5 × 106 cells were washed, resuspended with Locke’s solution (154 mM NaCl, 5.6 mM KCl, 1.2 mM MgCl2, 2.2 mM CaCl2, 5 mM HEPES, and 10 mM glucose, pH 7.3), and placed into a cuvette; and 40 μM cytochrome c was added. After a 1-min incubation, stimulants were added, and the change in absorbance at 550 nm was monitored. Superoxide dismutase was used as the control, setting the maximal value of the superoxide-mediated absorbance change. Calibration of the change in absorbance in terms of superoxide production was performed using the following equation: [superoxide] = AΔA/vIl/vIl/cells, where AΔA is the change in absorbance, v is the reaction volume, l is the time, K is the extinction coefficient for the difference between the light absorption of reduced cytochrome c and that of oxidized cytochrome c (21 × 105 cm−1 M−1), and I is the length of the cuvette.

Measurement of intracellular superoxide generation

The production of intracellular superoxides was determined based on the changes in fluorescence of DCFH-DA, an oxidation-sensitive fluorescence probe, with a slight modification of a previously published procedure (28, 29). Briefly, the cell suspension was incubated in fresh serum-free RPMI 1640 medium with 2 μM DCFH-DA at 37°C for 40 min under continuous stirring. The loaded cells were then washed twice with Locke’s solution. Then 2 × 106 cells were placed into a cuvette and in a thermostatically controlled cell holder at 37°C and continuously stirred. Fluorescence was excited at 488 nm, and emission was recorded at 530 nm. The change in fluorescence intensity was monitored.

Measurement of cytosolic Ca2+ concentration ([Ca2+]i)

The Mn2+ quenching of fura-2 fluorescence

The Mn2+ quenching assay was performed as described by Lee et al. (32) to measure the influx of Ca2+ from the extracellular space. Briefly, fura-2-loaded cells (5 × 106 cells/ml; described above) were placed into a quartz cuvette in a thermostatically controlled cell holder at 37°C under continuous stirring. Fluorescence was excited at 360 nm, i.e., the isosbestic wavelength at which Ca2+ does not affect fura-2 fluorescence and at which, therefore, changes are caused by Mn2+ quenching. Emission was recorded at 500 nm. The potency and slope of the change in fluorescence intensity were recorded after applying 2 mM MnCl2 and the drugs to be tested.

Measurement of InsP3 production

InsP3 mobilization was determined by competition assay of [3H]InsP3 for binding protein as described previously (33). To determine InsP3 production, 1 × 106 cells were stimulated with the drugs to be tested. The reaction was terminated by the addition of ice-cold 5% TCA containing 10 mM EGTA. The supernatant of the lysate was then saved and extracted with diethyl ether to remove TCA. The aqueous fraction after a final extraction was neutralized with 200 mM Trizma base to adjust it to pH 7.4. Twenty milliliters of extract was added to 20 ml of assay buffer (0.1 M Tris buffer containing 4 mM EDTA) and 20 ml of [3H]InsP3 (100 nCi/ml). The mixture was incubated for 15 min on ice and then centrifuged at 2000 × g for 10 min. Water (100 ml) and 1 ml of liquid scintillation cocktail were added to the pellet to measure the radioactivity. The InsP3 concentration of the sample was determined by comparison to a standard curve and expressed as picomoles per milligram of protein. The total cellular protein concentration was measured using the Bradford method after sonication of 1 × 106 cells.

Analysis of data

All quantitative data are expressed as the mean ± SEM. We calculated the IC50 with the Microcal Origin for Windows program. Differences were considered significant only for p < 0.05.

Results

We studied the effect of capsaicin on PAF-induced superoxide production and the increase in [Ca2+]i, in human promyelocyte HL-60 cells. HL-60 cells have served as a good model in which to study signal transduction of various receptors involved in the inflammatory processes of leukocytes (34). HL-60 cells express the PAF receptor, and its level of expression increases during granulocytic differentiation (35). As shown in Fig. 1A, PAF triggered

**FIGURE 1.** Effects of capsaicin on PAF-, extracellular ATP-, and fMLP-induced superoxide production in granulocytic differentiated HL-60 cells. Cells were preincubated with or without capsaicin using the indicated concentrations for 3 min, then treated with 300 nM PAF (A), 300 μM ATP (B), 3 μM fMLP (C), or 1 μM PMA (D) for 30 min. Superoxide formation was measured as described in Materials and Methods. Each result is the mean ± SEM of triplicate assays. The experiments were performed four times independently, and the results were reproducible.
differentiated HL-60 cells to secrete superoxide into the extracellular space. Under the above conditions, addition of capsaicin attenuated the production of superoxide in a concentration-dependent manner. It has been reported that PAF activates PLC and increases cytosolic Ca$^{2+}$ levels. We, therefore, tested the effect of capsaicin on the responses mediated by other PLC-coupled receptors in HL-60 cells. Capsaicin also inhibited superoxide production induced by fMLP (Fig. 1B) and extracellular ATP (Fig. 1C) with a similar inhibitory potency as that seen in the PAF response. However, PMA-induced superoxide production was not affected by capsaicin (Fig. 1D). The results suggest that capsaicin acts on the Ca$^{2+}$ response in the PLC signaling pathway and not on PKC.

It has been reported that the incubation of HL-60 cells with $>$100 μM capsaicin induces changes in the cytoskeleton and apoptosis (36). When we challenged cells with 300 μM capsaicin, we observed a slow, but prolonged, Ca$^{2+}$ rise, even though the increase was small, i.e., $<$100 nM (data not shown). The capsaicin-induced elevation of cytosolic Ca$^{2+}$ under the above conditions resulted from influx of Ca$^{2+}$ from the extracellular space and the release of Ca$^{2+}$ from the intracellular Ca$^{2+}$ pool. We suggest that this is not due to the activation of vanilloid receptors but, rather, to plasma and microsomal membrane perturbations that may occur and, upon prolonged Ca$^{2+}$ influx, drive the cell to apoptosis. Up to 100 μM capsaicin by itself did not trigger any cytosolic Ca$^{2+}$ rise.

When we added capsaicin, PAF-induced increases in cytosolic Ca$^{2+}$ were inhibited in undifferentiated HL-60 cells (Fig. 2A). The inhibition was more obvious in the sustained Ca$^{2+}$ level rather than the peak level. Capsaicin’s inhibitory effect disappeared in the absence of extracellular Ca$^{2+}$ using Ca$^{2+}$-free Locke’s solution (156.2 mM NaCl, 5.6 mM KCl, 1.2 mM MgCl$_2$, 5 mM HEPES, and 10 mM glucose, pH 7.3), but became prominent again when extracellular 2.2 mM Ca$^{2+}$ was reintroduced (Fig. 2B). The results, therefore, suggest that capsaicin inhibits SOCE-mediated by PAF.

The Ca$^{2+}$ responses to PAF were more dramatic in granulocytic HL-60 cells differentiated by incubation with 1 μM all-trans-retinoic acid for 5 days, but the characteristics of the signal transduction were same (data not shown).

When cytosolic Ca$^{2+}$ was elevated by the activation of other PLC-coupled receptors with fMLP or extracellular ATP, capsaicin inhibited the Ca$^{2+}$ increase in a similar manner as the PAF-induced Ca$^{2+}$ increase (data not shown), suggesting that capsaicin acted as a PLC inhibitor. We therefore tested whether capsaicin directly inhibited PLC by measuring the capsaicin effect on InsP$_3$ production induced by PAF, fMLP, and extracellular ATP. Capsaicin did not inhibit InsP$_3$ production induced by the above agents in undifferentiated (Fig. 3A) and differentiated granulocytic HL-60 cells (Fig. 3B). The results, therefore, reveal that capsaicin is not a PLC inhibitor.

Thapsigargin inhibits microsomal Ca$^{2+}$-ATPase, depletes intracellular Ca$^{2+}$ pools, and induces Ca$^{2+}$ influx of the SOCE kind. Capsaicin inhibited the thapsigargin-induced increase in cytosolic Ca$^{2+}$ (Fig. 4, A and B) in a concentration-dependent manner with an IC$_{50}$ of 24.8 ± 2.4 μM (Fig. 4C). To confirm the inhibitory effect of capsaicin on SOCE, we tested the thapsigargin-evoked influx of Ba$^{2+}$ and Mn$^{2+}$ ions that are experimentally added to the extracellular space to monitor the influx of Ca$^{2+}$ separate from the store-operated release. Capsaicin inhibited the fluorescence changes induced by the influx of Ba$^{2+}$ (Fig. 5A). Capsaicin also decreased the rate of fluorescence quenching caused by the binding of cytosolic fura-2 to Mn$^{2+}$ entering from the extracellular space (Fig. 5B). The data thus consistently indicated that the target site of capsaicin was the Ca$^{2+}$ influx through store-operated channels.

![FIGURE 2](http://www.jimmunol.org/)  
**FIGURE 2.** Effect of capsaicin on PAF-induced [Ca$^{2+}$], rise in HL-60 cells. A. Fura-2-loaded cells were challenged with 300 nM PAF (right) in the presence (dotted trace) or the absence (continuous trace) of 30 μM capsaicin (Cap). The cytosolic Ca$^{2+}$ concentration was measured as described in Materials and Methods. Typical Ca$^{2+}$ traces from more than three separate experiments are presented. The results were reproducible. B. The same experiment was performed in the absence of extracellular calcium before the addition of 4 mM CaCl$_2$ (Ca$^{2+}$). Typical Ca$^{2+}$ traces obtained in more than three separate experiments are presented. The results were reproducible.

![FIGURE 3](http://www.jimmunol.org/)  
**FIGURE 3.** Effects of capsaicin on PAF-, fMLP-, and extracellular ATP-induced InsP$_3$ generation in HL-60 cells. Cells were preincubated with (□) or without (■) 100 μM capsaicin for 3 min, then treated with 300 nM PAF, 1 μM fMLP, or 300 μM ATP for 15 s in undifferentiated (A) and all-trans retinoic acid-induced granulocytic differentiated HL-60 cells (B). The production of InsP$_3$ was measured as described in Materials and Methods. Each result is the mean ± SEM of triplicate assays. The experiment was performed three times independently, and the results were reproducible.
suggests that external Ca\(^{2+}\) oxide (Fig. 7A) as previously reported by Gallois et al. (38). This
stimulon-induced Ca\(^{2+}\) of SOCE (37). Fig. 6 shows that SK&F96365 decreased the thap-
site in the PLC signaling pathway. Activation of PLC leads to an
increase in intracellular Ca\(^{2+}\) and activates PKC. It is generally
results thus demonstrate that SK&F96365 and capsaicin exert the
same effect on superoxide production.

There still remained the possibility that capsaicin acted on va-
niloid receptors. We therefore tested capsaicin in combination
with an agonist and an antagonist of vanilloid receptors. Resinif-
eratoxin, which is a concentration 100 times higher than that gen-
erally used, an inhibitory effect on the thapsigargin-induced SOCE
was not detectable (Fig. 8). It has been reported that ruthenium
red and capsazepine act on the vanilloid receptor as antagonists
(40). Pretreatment of cells with 10 \(\mu\)M ruthenium red did not block
the capsaicin-inhibited production of the thapsigargin-induced SOCE
(Fig. 8B). Finally, capsazepine had a similar effect on the inhibi-
tion of the thapsigargin-induced SOCE as capsaicin (Fig. 8C).

Discussion
In the present study we demonstrate that capsaicin inhibits PAF-
mediated superoxide production. Many inflammatory signals, in-
cluding PAF, trigger receptor-mediated PLC activation as part of
their signaling mechanism. Our experiments with FMLP and ex-
tracellular ATP, which activate PLC-coupled receptors (Fig. 1),
suggested that the capsaicin-mediated inhibition of PAF-induced
superoxide production was mediated by blockage of a common
site in the PLC signaling pathway. Activation of PLC leads to an
increase in intracellular Ca\(^{2+}\) and activates PKC. It is generally

To study the capsaicin effect on inflammatory reactions, we
monitored the effect of SOCE on superoxide formation in granu-
locytic differentiated HL-60 cells. PAF prominently evoked the
formation of cytosolic superoxide; however, removal of extracel-
ular Ca\(^{2+}\) dramatically reduced the production of cytosolic super-
oxide (Fig. 7A) as previously reported by Gallois et al. (38). This
suggests that external Ca\(^{2+}\) entry significantly contributes to the
formation of superoxides. When cells were treated with capsaicin,
the PAF-induced cytosolic superoxide production was clearly
diminished (Fig. 7B). SK&F96365 decreased the PAF-induced su-
peroxide formation just a little more potently than capsaicin. The

To further assess the target site of capsaicin, we compared the
actions of capsaicin and SK&F96365, which is a known antagonist
of SOCE (37). Fig. 6 shows that SK&F96365 decreased the thapsi-
gargin-induced elevation of \([\text{Ca}^{2+}]\), and that the successive ad-
dition of capsaicin did not add to the inhibition of the sustained
Ca\(^{2+}\) level without capsaicin treatment. Each point was
obtained from triplicate experiments and is the mean \(\pm\) SEM. The results
were reproducible.

![FIGURE 4. Effect of capsaicin on thapsigargin-induced SOCE in HL-60 cells. A, Fura-2-loaded cells were treated with the indicated concentrations of capsaicin (Cap), then challenged with 1 \(\mu\)M thapsigargin (TG). Stimuli given are as follows: vehicle (a), 30 \(\mu\)M capsaicin (b), and 50 \(\mu\)M capsaicin (c). B, Fura-2-loaded cells were treated with the indicated concentrations of capsaicin (Cap) after incubation with 1 \(\mu\)M thapsigargin (TG). Stimuli given are as follows: vehicle (a), 10 \(\mu\)M capsaicin (b), 50 \(\mu\)M capsaicin (c), and 100 \(\mu\)M capsaicin (d). C, Concentration-dependent effect of capsaicin on thapsigargin-induced SOCE. The same experiment shown in B was performed with various concentrations of capsaicin. Net decreases in \([\text{Ca}^{2+}]\), are depicted as a percentage of the control value (thapsigargin-induced Ca\(^{2+}\) level without capsaicin treatment). Each point was obtained from triplicate experiments and is the mean \(\pm\) SEM. The results were reproducible.](http://www.jimmunol.org/Downloaded from http://www.jimmunol.org/)

![FIGURE 5. Effect of capsaicin on thapsigargin-induced Ba\(^{2+}\) and Mn\(^{2+}\) influx in HL-60 cells. A, Fura-2-loaded cells were stimulated with 1 \(\mu\)M thapsigargin (TG) with or without the preincubation of capsaicin for 3 min in Ca\(^{2+}\)-free medium, then 5 mM Ba\(^{2+}\) was added. Stimuli given are as follows: vehicle (a), 30 \(\mu\)M capsaicin (b), and 50 \(\mu\)M capsaicin. The results are depicted as fluorescence ratio of 340 nm and 380 nm \(\left(F_{340}/F_{380}\right)\). The experiments were independently conducted more than five times. The results were reproducible. B, Mn\(^{2+}\)-induced fura-2 fluorescence quenching was recorded in fura-2/AM-preloaded cells incubated with 1 mM Mn\(^{2+}\) and drugs at the indicated point (arrow). Stimuli given are as follows: vehicle (a), 1 \(\mu\)M thapsigargin with 100 \(\mu\)M capsaicin (b), and 1 \(\mu\)M thapsigargin (c). The influx of Mn\(^{2+}\) was measured as described in Materials and Methods. The results are depicted as fluorescence intensities at 360 nm \(\left(F_{360}\right)\). The data presented are representative of four independent experiments, and the results were reproducible.](http://www.jimmunol.org/Downloaded from http://www.jimmunol.org/)
accepted that the Ca\(^{2+}\) increase and PKC activation are synergistically involved in superoxide production (41). Interestingly, capsaicin did not inhibit PMA-induced superoxide production. This indicates that capsaicin does not directly inhibit PKC.

PLC-mediated cytosolic Ca\(^{2+}\) elevation is achieved by Ca\(^{2+}\) release from InsP\(_3\)-sensitive stores and subsequent Ca\(^{2+}\) influx from the extracellular space, the so-called SOCE, which is activated by the depletion of intracellular Ca\(^{2+}\) stores (42). SOCE is thought to be a major regulator of immune responses, including \(O_2^\cdot\) production in granulocytic differentiated HL-60 cells (37) and neutrophils (43), IL-8 release in neutrophils (44), histamine release in mast cells (45), and platelet aggregation (46). It has been reported that the PAF-induced priming of neutrophils requires Ca\(^{2+}\) influx (47), which has been found to be SOCE (24). Our results reveal that capsaicin inhibited Ca\(^{2+}\)-sensitive superoxide production (Figs. 1 and 7) and Ca\(^{2+}\) influx, which is activated subsequent to the depletion of Ca\(^{2+}\) stores (Figs. 4 and 5) and is affected by SK&F 96365, a SOCE inhibitor (Fig. 6), whereas capsaicin did not directly inhibit PLC or the InsP\(_3\)-sensitive Ca\(^{2+}\) release (Figs. 2 and 3). We thus can conclude that capsaicin-induced inhibition of SOCE will result in a reduction of PAF-induced superoxide formation in HL-60 cells.

**FIGURE 6.** Effect of SK\&F96365 on the inhibitory effect of capsaicin toward thapsigargin-induced SOCE. A, Fura-2-loaded HL-60 cells were treated with 1 \(\mu\)M thapsigargin (TG) and then challenged with 100 \(\mu\)M capsaicin (Cap) in the presence of 10 \(\mu\)M SK\&F96365 (SKF). B, Cells were treated with 1 \(\mu\)M thapsigargin (TG), then challenged with 10 \(\mu\)M SK\&F96365 (SKF) in the presence of 100 \(\mu\)M capsaicin (Cap). The presented data are representative of more than five independent experiments. The results were reproducible.

**FIGURE 7.** Capsaicin inhibits intracellular superoxide generation by blocking Ca\(^{2+}\) influx. A, DCFH-loaded cells were treated with 3 \(\mu\)M PAF in the presence (a) or the absence (b) of 2.2 mM extracellular free Ca\(^{2+}\). Changes in fluorescence intensity were monitored. Fluorescence intensities at 488 nm (F\(_{488}\)) are depicted. Cytosolic superoxide production was measured as described in Materials and Methods. The experiments were independently conducted more than three times, and the results were reproducible. B, DCFH-loaded cells were preincubated with drugs at the indicated point (Pre) and then treated with 3 \(\mu\)M PAF. Changes in fluorescence intensity were monitored. Stimuli given are as follows: vehicle (a), 50 \(\mu\)M capsaicin (b), and 10 \(\mu\)M SK&K96365 (c). The presented data are representative of three independent experiments, and the results were reproducible.

**FIGURE 8.** The effects of resiniferatoxin, ruthenium red, and capsazepine on the capsaicin-evoked inhibition of thapsigargin-induced [Ca\(^{2+}\)]\(_i\) rise in HL-60 cells. A, After pretreatment with 1 \(\mu\)M thapsigargin (TG), fura-2-loaded cells were challenged with 100 \(\mu\)M capsaicin (Cap) in the presence of 1 \(\mu\)M resiniferatoxin (Res). B, Cells were treated with 1 \(\mu\)M thapsigargin, then challenged with 100 \(\mu\)M capsaicin in the presence of 10 \(\mu\)M ruthenium red (RR). C, Cells were treated with 30 \(\mu\)M capsazepine (Capz) after incubation with 1 \(\mu\)M thapsigargin. All presented data are typical Ca\(^{2+}\) traces of more than five separate experiments. The results were reproducible.
In this report we demonstrate that capsaicin directly acts on immune cells attenuating their inflammatory responses in addition to their effect on afferent nerves. There was evidence presented in a previous report that capsaicin could inhibit the production of superoxides by macrophages in the absence of afferent neuron fibers (21). Capsaicin had been thought to exclusively have an effect on the desensitization of neurogenic inflammation. However, we carefully suggest the possibility that the direct inhibition of superoxide formation by capsaicin could be another important aspect in the alleviation of inflammation. The capsaicin concentrations used for the blockage of neurogenic inflammation are 25–100 mg/kg (17–19), which roughly equals 1–5 mM and therefore could be enough to directly block the inflammatory action of immune cells. It is known that vanilloid receptors are exclusively expressed on afferent nerves. Although vanilloid receptors were detected on murine mast cells (48), there is no evidence of expression of vanilloid receptors on other immune cells such as monocytes, macrophages, or neutrophils. The capsaicin-induced effects could be classified into two different categories: vanilloid receptor-mediated responses and nonvanilloid type responses. Our findings suggest that the capsaicin effect is of the nonvanilloid type because of 1) its high effective concentration needed for the effect (Fig. 1), 2) the lack of antagonistic effect of a classical vanilloid agonist, such as resiniferatoxin (Fig. 8A), and 3) no detectable antagonistic effect of classical vanilloid antagonists, such as capsazepine and ruthenium red (Fig. 8, B and C). Interestingly, capsazepine also inhibited SOCE just as capsaicin. Our results correlate with other studies that have seen effective concentrations of capsaicin in the micromolar range (17, 18), whereas vanilloid receptor can be activated with concentrations in the nanomolar range. Our results also agree with reports of some capsaicin-mediated effects not correlating with typical features of the unusual responses of vanilloid receptors to vanilloid antagonists (49).

We studied the inhibitory effect of capsaicin on PAF-induced superoxide formation. Because PAF is a potent inducer of inflammation, a PAF antagonist could be a promising agent for therapeutic applications that treat inflammation, although clinically available drugs are still at the developmental stage. Our results provide a first clue toward understanding of the capsaicin anti-inflammatory effect as it inhibits PAF-mediated reactions.

Acknowledgments

We thank G. Hoschek for editing this manuscript.

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