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Capsaicin effects on non-neuronal plasma membranes

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Capsaicin has been touted as a pharmacological tool specific for sensory afferent neurons and is widely used in neurophysiological studies. However, we have recently demonstrated that in concentrations commonly employed within the gastrointestinal tract, capsaicin inhibits platelet aggregation to at least three different stimuli. Since this was observed in a nerve free system it raised the question of how specific capsaicin is. In this communication we report that capsaicin has profound effects on physical properties of non-neuronal cell plasma membranes. These effects were observed while measuring the effect of capsaicin upon the fluidity of both intact cell membranes and a variety of purified membrane preparations. Membrane fluidity was assessed with the fluorescent probes diphenylhexatriene (DPH) and its trimethylamino derivative TMA-DPH and demonstrated concentration-dependent capsaicin effects. Furthermore, the effects were cell specific and for full expression required both intact cells and a non-lipid extractable component of the plasma membrane. These non-neuronal effects must be carefully considered when contemplating the explanation for capsaicin-induced effects.

Introduction

Capsaicin is the major pungent ingredient of hot red peppers from the plant genus *Capsicum*. It's widespread use as a pharmacological tool derives from the belief that capsaicin is selective in stimulating sensory afferent neurons [1]. Thus, when used in physiological systems, even those containing many different cell types, observed effects are attributed to specific neuronal activation. For example, within the gastrointestinal tract perfusing the stomach with solutions containing capsaicin in concentrations from 25 to 640 μM has been shown to increase gastric mucosal blood flow [2] and to protect the stomach from ulcerogenic agents [3]. These effects were attributed to involvement of sensory afferent neurons despite the presence of many other cell types that could potentially mediate such interactions. The mechanism underlying the neuronal effects of capsaicin is unclear but it has been suggested that this lipophilic compound may insert into the cell plasma membrane and by altering membrane fluidity impair ionic fluxes [4]. Since this mechanism would not be expected to result in specificity for neurons we recently examined the effects of capsaicin on platelet aggregation [5]. We were able to demonstrate that in a nerve free system capsaicin potently inhibited platelet aggregation when added in a fashion similar to that employed in the gastrointestinal tract. Thus, if capsaicin worked via the proposed mechanism it appeared that platelets, in addition to neurons, were amenable to its effects. The present study was performed to address several questions. Firstly, since capsaicin inhibits platelet aggregation does it also fluidize platelet plasma membranes? This relationship might not be unexpected since it has been known for some time that fluidization of the platelet plasma membrane decreases platelet aggregation [6,7]. Finally, if capsaicin fluidized platelet plasma membranes was this specific for platelets (and presumably nerves) or alternatively are other cell or artificial membrane systems affected in a similar fashion? In this communication we report evidence suggesting that capsaicin does fluidize platelet plasma membrane, under these conditions, but does not appear to be a general membrane fluidizer such as benzyl alcohol. Its effects appeared to be cell specific and in certain cells both a cytoplasmic and membrane component appeared necessary for full expression of the effect.

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Methods

Chemicals

All fluorescent probes were purchased from Molecular Probes (Junction City, OR), and stored in ethanol or dimethylsulfoxide (DMSO). All other reagents were supplied by Sigma and were of the highest purity available. Capsaicin was dissolved in DMSO at concentrations ranging from 1 to 32 mM and added directly to cell or membrane preparations such that final concentrations ranged from 10 to 320 μM while DMSO concentrations remained constant at 1%. This concentration of DMSO was always added to the blank samples. A variety of cell types were examined in these studies. Unless specified otherwise cells were purified and studied immediately.

Platelets

Rabbit washed platelets were isolated and purified in the presence of prostacyclin (PGI_2; 300 ng/ml) as described previously [8]. Indomethacin (10 μg/ml) in 1.25% sodium bicarbonate was added to the final platelet rich solution so as to ensure the inhibition of platelet formation of pro-aggregatory cyclo-oxygenase products. The platelet solution was allowed to stand at 22°C for 1.5 h to permit degradation of prostacyclin. Platelet counts varied between 10^7–10^9/ml of platelet solution.

B-Lymphocytes

Tissues from the ileal Peyer's patch of a 10-week-old lamb were placed in ice-cold RPMI and single cell suspensions were prepared according to published procedures [9]. The resulting B-lymphocyte suspension contained $> 10^8$ cells/ml of which 97–100% were viable and were of > 95% purity as assessed by flow cytometry.

Peritoneal mas cells

Male, Sprague-Dawley rats (250–350 g) were etherized and killed by cervical dislocation. Peritoneal cells were then obtained through lavage of the peritoneal cavity with 15 ml of cold (4°C) Hepes-buffered Tyrode's solution containing 0.1% BSA. Peritoneal mast cells were recovered from a layered two-step discontinuous Percoll gradient (Pharmacia Ltd., Uppsala, Sweden) as described previously [10]. Peritoneal mast cell purity ranged from 97.99% and viability was approx. 97%.

Whole cell labelling

Washed platelets were labelled immediately with either DPH or TMA-DPH by the methods described by Kitagawa et al. [6]. Mast cells and Peyers patch lymphocytes were also labelled immediately following isolation by the method of Kuhry et al. [11]. In preliminary experiments utilizing peritoneal mast cells and TMA-DPH maximal fluorescence was observed within 5 min following addition of probe. Since this probe remains localized to the plasma membrane for a limited period of time all studies in whole cells were performed between 5 and 10 min following the labelling procedure [20].

Erythrocyte preparation and labelling

Following an overnight fast, blood samples were collected from human volunteers into vacutainer tubes containing Acid-Citrate-Dextrose as a preservative (Becton-Dickinson, Rutherford, NJ), and stored at 4°C for 1–3 h. The blood was washed with phosphate-buffered saline (mM) (NaCl (145); KCl (5); Na_2PO_4 (5); pH 7.4) and filtered through cotton wool to remove platelets and leucocytes followed by gentle centrifugation for 10 min [12]. The sample was washed and centrifuged twice more and any remaining buffy coat aspirated with the supernatant. Erythrocytes were then suspended to a haematocrit of 1% and fluorescent probe added. The fluorescent probes used were diphenylhexatriene (DPH) or its trimethylammonium derivative (TMA-DPH). All probes were stored, protected from light at −20°C, in ethanol and were added to the erythrocyte preparation to give a final probe concentration of $5 \cdot 10^{-6}$ M with a final ethanol concentration of 0.5%, according to the method of Schacter [13]. After incubation for 10–15 min at 37°C, the erythrocytes were gently pelleted and the supernatant discarded. The pellet was washed twice more with approximately 25 volumes of PBS. Labelled erythrocytes were resuspended to a haematocrit of 0.025% in PBS. For certain experiments artificial liposomes were constructed utilizing the method of Scotto et al. [14]. Liposome lipid composition consisted of 60% dimyristylphosphatidylcholine and 40% cholesterol (wt%) or isolated lipids from erythrocyte ghosts. In each case fluorescent probe was added to the lipid following formation of the liposome, in order to as closely simulate the situation with intact cells/membrane as possible. Ghosts were .prepared according to the method of Zachariasse et al. [15] and the lipids subsequently extracted by the method of Folch [16].

Enterocyte microvillus membrane isolation and labelling

New Zealand White rabbits, weighing between 0.75 and 1.0 kg, were killed with an overdose of intravenous pentobarbitol. The jejunum was rapidly removed and flushed with ice-cold PBS. The mucosal surface was carefully scraped with a glass slide and microvillus membranes prepared using the Ca^{2+} precipitation method described by Kessler [22]. The final membrane pellet was suspended in a buffer containing 100 mM KCl, 10 mM Hepes, 10 mM Tris and stored in liquid nitrogen [23]. Sucrase activity of membrane isolates and their homogenates was determined by the method

of Dahlqvist [24]. The resulting membrane preparations consistently had a 20-fold increase in the specific activity of sucrase per mg of protein with no concentration of Na^+,K^+-ATPase activity over the activity of the starting homogenate (data not shown). These methods have been previously described in detail from our laboratory [25]. Following a gentle thawing at room temperature vesicles were resuspended in the original buffer with a 21 guage syringe and labelled with either DPH or TMA-DPH as described previously.

Steady-state fluorescence measurements

The term membrane fluidity is used in this paper to denote the relative motional freedom of molecules in a lipid bilayer. A more complete description and the implications of this measurement is given elsewhere [17,18]. Steady-state fluorescence polarization of the probes DPH and TMA-DPH were measured in an SPF-500C fluorometer specifically adapted for polarization work (SLM Aminco). Emission maxima were observed at 450 nm and a bandpass of 10 nm was used for both the excitation and emission monochromaters. A 420 nm sharp cutoff filter was placed in the emmission lightpath to eliminate scattered light. Data are reported as the maximal steady-state anisotropy parameter observed with progressive dilutions of cell or membrane suspensions. In all cases a cell concentration could be obtained such that a 10-fold dilution did not result in a significant increase in the anisotropy parameter. At this point loss of polarization secondary to particle scattering was considered minimal [19]. In all experiments total fluorescence was determined in order to evaluate the possibility that fluorescent lifetime was altered. This parameter cannot be directly measured with steady-state methodology, however, since fluorescent lifetime is directly proportional to total fluorescence a constant total fluorescence for a constant amount of probe and membrane is evidence that lifetime did not change. The probes were selected since each reports from a unique position in the bilayer. TMA-DPH with its cationic headgroup remains localized to the plasma membrane over short periods of time and remains relatively near the phospholipid headgroups of the external hemileaflet [20]. Conversely DPH appears to report on conditions nearer the core of the bilayer.

Fluidity data are reported as the steady-state anisotropy parameter rather than the limiting hindered anisotropy. Although the latter can be calculated from the former on the basis of an empirical relationship [21] this could only be done for the probe DPH. In order to present data from both probes for each membrane in the same figure we simply present the steady-state anisotropy parameter.

Throughout this paper we refer to the amount of capsaicin added to the samples in terms of concentration. This has been done for several reasons, an important one being consistency with the work previously published [2,3,5]. However, the preferable units would be the molar amount of capsaicin relative to the molar amount of membrane lipid and fluorescent probe present in each sample. Unfortunately when working with whole cells that possess intracellular membranes into which some probes partition and some don't it becomes virtually impossible to present reliable data on the probe/lipid/capsaicin ratios in each membrane of interest. This is of particular importance for the probe TMA-DPH. For systems that involved only an isolated membrane or red cells (that were assumed to have no significant intracellular membranes) we routinely calculated these ratios. Lipid/probe ratios (mol/mol) varied between 1500 and 2000. All fluorescence determinations were performed in a 3 ml cuvette and the final concentration of capsaicin in the buffer adjusted to simulate the concentrations previously utilized [5]. This meant that relatively large amounts of capsaicin were available per mole of lipid. At the lowest concentration of capsaicin used (40 μM) the capsaicin/lipid molar ratios for microvillus membrane, liposomes and erythrocytes were 1.43, 0.14 and 0.33, respectively. The amount of lipid in the plasma membrane of mast cells and lymphocytes cannot be quantitated without purifying these membrane preparations, therefore, data for these preparations cannot be given. However, since the light scattering properties of these suspensions were virtually identical to the red cell preparations they would be expected to be similar.

Statistical analysis

All data are reported as the mean ± S.E. for quadruplicate determinations in at least four sets of cells or membrane preparations. Differences between groups were compared using ANOVA with a Tukey test for post hoc comparisons. Calculations were performed using a commercial statistics package, Systat (Evanston, IL). A value of $P < 0.05$ was considered significant.

Results

Two fluorescent probes were utilized to evaluate membrane physical properties of purified platelets. TMA-DPH localizes to the plasma membrane of cells and as illustrated in Fig. 1 we observed a significant dose-dependent decrease in the anisotropy parameter for this probe with increasing capsaicin concentration. The dotted lines encompass the 95% confidence intervals for measurements obtained with platelets incubated with vehicle alone. Clearly, with even the lowest capsaicin concentration employed, 40 μM, a significant reduction in this parameter was evident. Similar find-

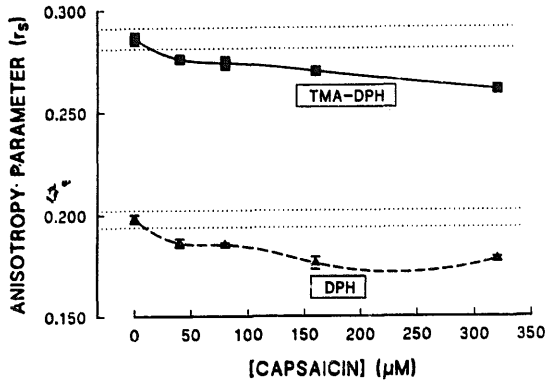

Fig. 1. Effect of capsaicin on washed rabbit platelets utilizing the probes DPH and TMA-DPH. Each point represents the mean ± S.E. for at least four separate platelet preparations with each measurement performed in quadruplicate. Dotted lines represent the 95% confidence intervals around the point with no added capsaicin. All other points were significantly different from this one.

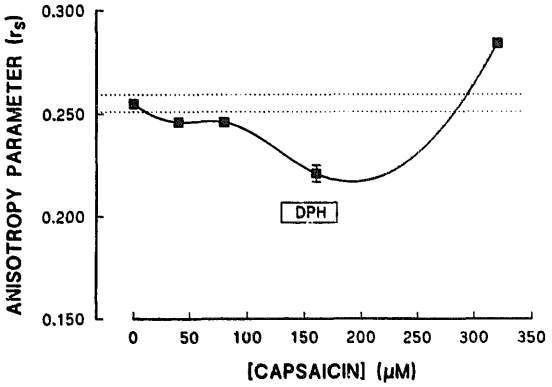

Fig. 3. Intact human erythrocytes obtained from six volunteers and separately labelled with DPH. Once again each point represents the mean ± S.E. and the dotted lines outline the 95% confidence interval of the control samples.

ings were apparent utilizing the probe DPH which localizes to both plasma and intracellular membranes.

Since certain actions of the enteric nervous system may involve mast cells located in the lamina propria of the intestinal wall we next proceeded to evaluate the effect of capsaicin on membrane physical properties of isolated peritoneal mast cells. Utilizing a similar experimental protocol mast cells were isolated and labelled with either TMA-DPH or DPH and subsequently exposed to varying concentrations of capsaicin (Fig. 2). For the probe TMA-DPH a biphasic action of capsaicin was noted. With low concentrations of capsaicin a decrease in anisotropy parameter, similar to that observed with platelets, was apparent. However, at a capsaicin concentration of 320 μM the anisotropy parameter increased to values significantly greater than those observed in the absence of this compound. For the probe DPH a similar increase with high capsaicin concentrations was also evident. However, since DPH

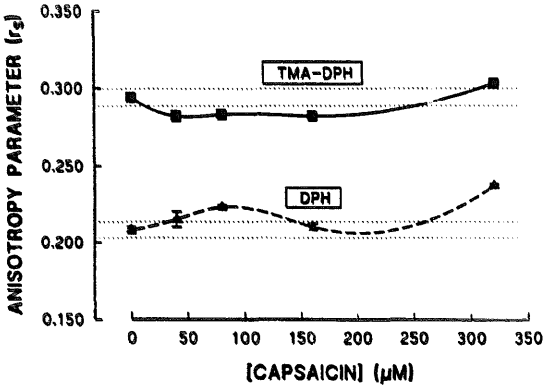

Fig. 2. Effect of capsaicin on peritoneal mast cells. Three separate isolates are shown with measurements performed as in Fig. 1. For TMA-DPH points lying outside the 95% confidence intervals were significantly different from controls while for DPH the addition of 320 μM capsaicin provided a significant increase in anisotropy parameter.

rapidly partitions into intracellular membranes the localization of this effect is difficult to ascertain.

In an attempt to circumvent these problems we isolated red cells and examined the effect of capsaicin in this cell population. The data obtained from these studies is presented in Fig. 3. Since the red cell has few if any internal cell membranes it represents an ideal cell for this type of study and DPH can be used to label the plasma membrane alone. Within this cell population capsaicin had a clear biphasic action on the plasma membrane. At low concentrations a marked increase in motional freedom of DPH was apparent (reduction in anisotropy parameter) while at 320 μM once again a significant decrease was observed. Thus, in three different cell types, platelets, peritoneal mast cells and finally the red blood cell, capsaicin has a concentration-dependent effect on the plasma membrane.

The mechanism(s) underlying these observations remained unclear from these experiments. Capsaicin could be acting as a non-specific membrane fluidizer, although the increase in anisotropy parameter observed in red cells and peritoneal mast cells would be difficult to explain. However, in order to address this possibility artificial liposomes were prepared from dimyristylphosphatidylcholine and cholesterol (60:40, w/w) and labelled with DPH. Fig. 4 illustrates the effect of capsaicin on these membrane preparations. Absolutely no fluidization was apparent over the concentration range utilized. In order to demonstrate that these vesicles could be fluidized they were subsequently exposed to A_2C or benzyl alcohol over the concentration range of 0–5 μM or 0–30 mM, respectively, with significant decreases in anisotropy parameter observed (data not shown). Thus, the fluidizing effect of capsaicin over this concentration range does not appear to be due to an intrinsic action on membrane lipids.

The next question we addressed was whether the intact cell was required to produce the capsaicin-in-

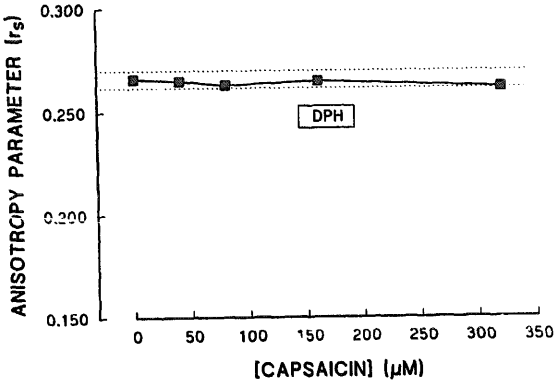

Fig. 4. Artificial liposomes prepared from acetone washed dimyristylphosphatidylcholine and cholesterol and labelled with DPH. Each values represents the mean ± S.E. for 4 separate preparations. No significant effect was noted for any concentration of capsaicin.

duced membrane effect. The red cell was the simplest cell available that provided a capsaicin effect and could be easily separated into a pure membrane fraction; therefore it was used for these studies. Fig. 5 illustrates the effect of capsaicin on the intact cell and on the same cell preparations following the production of red cell ghosts. It is apparent that in the absence of capsaicin membrane physical properties of the intact cell and ghost are indistinguishable suggesting that little disruption of membrane architecture occurred in the preparation of a purified plasma membrane fraction. However, the response to capsaicin was radically different between the two preparations. In the intact cell capsaicin produced a biphasic response in membrane physical properties while in the isolated plasma membrane a monotonous increase in motional freedom of

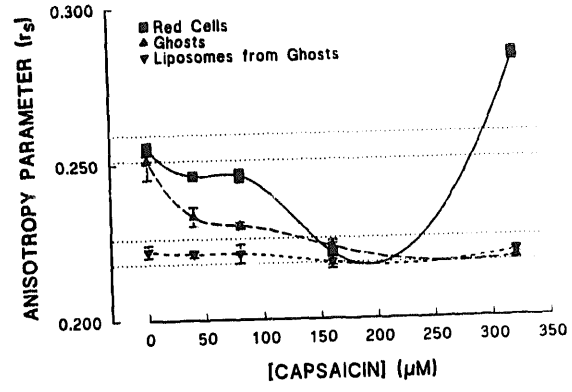

Fig. 5. Effect of capsaicin on various erythrocyte fractions. In the solid squares are the intact erythrocyte data previously shown in Fig. 3. They are included here for comparison. In triangles are four of these preparations from which membrane ghosts were prepared. With increasing capsaicin concentrations a significant reduction in anisotropy parameter was evident. For three separate erythrocyte preparations membrane lipids were Folch-extracted from the ghost membrane preparations and used for the preparation of liposomes. Data from these membrane preparations is shown with the inverted triangles. The lower dotted lines represent the 95% confidence intervals for these liposomes with no added capsaicin. It was apparent that capsaicin had no effect in this preparation.

DPH was observed. Thus, the decrease in motional freedom of the plasma membrane induced by high concentrations of capsaicin requires the intact cell and presumably a cytoplasmic component. From our experiments with artificial liposomes it would be predicted that red cell membrane lipids would be unresponsive to capsaicin. However, to ensure that this was indeed the case we extracted membrane lipids from the ghosts and prepared liposomes. As illustrated in Fig. 5 capsaicin did not alter the physical properties of these membranes. The lower dotted lines represent the 95% confidence intervals for membrane physical properties in erythrocyte membrane lipid in the absence of capsaicin. The difference between the values seen in this preparation and those of the intact cell or ghost represent the contribution of membrane proteins to membrane physical properties. It is also apparent that the responsiveness of this membrane preparation to capsaicin is entirely dependent on a component removed in the lipid extraction, presumably a protein.

Thus, it appeared that the responsiveness of cells to capsaicin, at least in terms of membrane fluidity, was mediated by a non-Folch extractable membrane component and required an intact cell system for full expression. With this being the case we predicted that we could find biological membrane systems in which capsaicin did not alter membrane physical properties over this range of concentrations. Figs. 6 and 7 reveal that this was indeed correct. In Fig. 7 the absence of effect of capsaicin on membrane physical properties in brush border membranes isolated from jejunal enterocytes is documented using the probe DPH. Fig. 6 illustrates the results of incubating capsaicin with B-lymphocytes isolated from Peyers patches. Since intact cells were once again used both TMA-DPH and DPH were used to label the cells. It is apparent from these data that the effect of capsaicin on membrane physical properties is indeed cell specific.

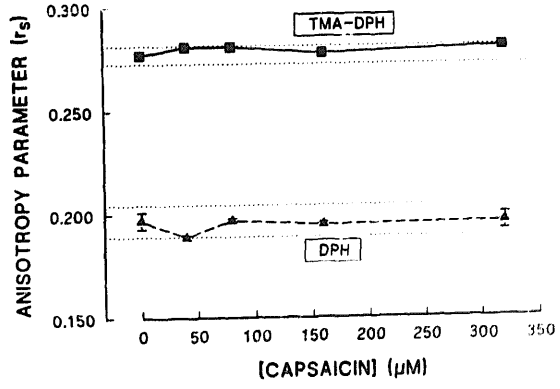

Fig. 6. Effect of capsaicin on B-lymphocytes isolated from sheep Peyers patches. The anisotropy parameter of either TMA-DPH or DPH was not affected by capsaicin over this concentration range. Each Point represents the mean ± S.E. from three separate preparations isolated from patches of a single animal.

TABLE I

Total fluorescence for DPH in membranes exposed to capsaicin

Values represent the mean ± S.E. for at least three samples determined in quadruplicate. Assays were performed with a constant number of cells or mass of lipid and probe under constant fluorometric conditions. Results are expressed in arbitrary fluorescence units. * $P < 0.05$ vs. value with no added capsaicin.

[Capsaicin] (μM)	Platelet	PMC	Red cell	Liposome	Red cell ghost	Red cell liposome	Lymphocyte	MVM
0	8.7±1.8	5.5±0.2	4.8±0.7	7.6±0.2	7.4±0.2	5.7±0.1	4.9±0.5	8.1±0.5
40	9.6±0.1	7.5±0.1 *	7.5±0.5 *	7.1±0.3	6.9±0.2	5.4±0.1	6.4±0.5	7.1±0.4
80	7.8±1.1	7.6±0.2 *	7.3±0.4 *	7.1±0.1	6.8±0.2	5.3±0.1	7.6±0.4 *	7.7±0.1
160	8.3±1.0	6.2±1.3	6.9±0.4	6.8±0.2	6.9±0.1	5.6±0.2	8.4±0.3 *	8.3±0.4
320	8.1±0.9	8.4±0.3 *	8.8±0.3 *	7.3±0.2	7.3±0.2	5.2±0.1	8.9±0.3 *	7.7±0.5

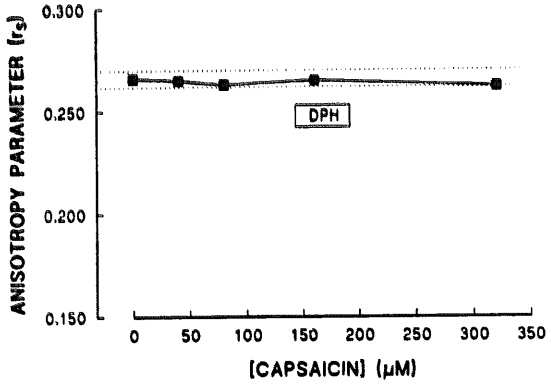

Fig. 7. Effect of capsaicin on rabbit jejunal microvillus membrane. Each point represents the mean ± S.E. from three separate membrane preparations. No significant effect of capsaicin was noted at any concentration.

To ensure that the effects of capsaicin noted on the steady-state anisotropy parameter could not be explained by an alteration in fluorescent lifetime of the probe we routinely measured the total fluorescence of each sample. These results, for the probes DPH and TMA-DPH, are presented in Tables I and II. In each case care was taken to ensure that constant amounts of probe and membrane or cell number were analyzed under identical fluorometric conditions. Under these conditions total fluorescence is directly proportional to the fluorescent lifetime of the probe. Thus, an increase in fluorescent lifetime could account for a decrease in the steady-state anisotropy parameter. For the probe TMA-DPH a significant increase in total fluorescence was never observed in any preparation. Furthermore, when isolated membranes or liposomes were utilized the same observation held true for DPH. However, in the whole cell preparations of peritoneal mast cells, red cells or lymphocytes an increase in DPH fluorescence was apparent with increasing capsaicin concentrations. In the case of PMC an increase in total fluorescence, and possibly fluorescent lifetimes, cannot be the explanation for the observed increase in anisotropy parameter. However, for the red cell with 40 μM capsaicin a reduction in anisotropy parameter was coupled with an increase in total fluorescence. This could be interpreted as being secondary to an alteration in fluorescent lifetime of the probe rather than a change in membrane physical properties per se. However, with further increases in capsaicin concentration the anisotropy parameter continued to fall significantly while total fluorescence remained constant. Thus, over this range of concentrations the effect of capsaicin on membrane physical properties is unlikely to be due to an unrecognized effect on fluorescent lifetime. For experiments involving lymphocytes increasing capsaicin concentrations produced a monotonous increase in the total fluorescence of DPH (but not TMA-DPH) while the anisotropy parameter remained constant.

TABLE II

Total fluorescence for TMA-DPH in membranes exposed to capsaicin

Values represent the mean ± S.E. for at least three samples determined in quadruplicate. Assays were performed with a constant number of cells or mass of lipid and probe under constant fluorometric conditions. Results are expressed in arbitrary fluorescence units. * $P < 0.05$ vs value with no added capsaicin.

[Capsaicin] (μM)	PMC	Platelet	Lymphocyte
0	5.1±0.5	6.2±0.5	6.4±0.2
40	6.4±0.6	6.7±0.4	5.7±0.2
80	5.9±0.8	6.4±0.3	6.4±0.3
160	6.3±0.6	6.7±0.3	5.8±0.2
320	7.5±0.6	7.3±0.6	7.1±0.3

Discussion

The utility of any pharmacological tool rests upon an appreciation of its specificity and sensitivity in the systems used. Capsaicin is widely regarded as being extremely specific as an activator of sensory afferent neurons [1,3,26,27]. However, in a previous study we have demonstrated that this agent significantly impairs the functional response of platelets to three different aggregating stimuli [5]. Since this observation was made

in a system composed strictly of isolated platelets it appears that the specificity of capsaicin may have been overstated. In the current study we have extended these original observations in an attempt to elucidate the mechanism by which capsaicin might impair platelet aggregation.

It has previously been demonstrated that agents fluidizing the platelet plasma membrane interfere with aggregation. Furthermore, since fluidizing agents also impair the accumulation of intracellular calcium induced by ADP or thrombin, interference with calcium fluxes across the plasma membrane has been postulated as a mechanism for these observations [7]. Capsaicin inhibits calcium-dependent platelet aggregation induced by these agents but does not interfere with the calcium-independent shape change observed with these agonists [5]. Thus, we postulated that, as a lipophilic agent, capsaicin might be a potent fluidizer of platelet plasma membranes. Over a concentration range that inhibited platelet aggregation capsaicin did indeed potently fluidize platelet membranes as assessed by two fluorescent probes. TMA-DPH localizes fairly specifically to the plasma membrane when used over a short time course as in this study while DPH rapidly partitions into intracellular membranes as well [28]. However, for both probes a concentration-dependent fluidization of platelet membranes was observed (Fig. 1). These results were not only significant but were also unlikely to be explained by alterations in the fluorescent lifetime of our probes. Using steady-state techniques it is impossible to directly quantitate fluorescent lifetimes. However, since total fluorescence for a constant amount of membrane and probe is directly proportional to fluorescent lifetime differences in the latter can be implied by alterations in the former. Since no significant differences were observed in total fluorescence it is unlikely that alterations in fluorescent lifetime occurred with the addition of capsaicin.

From these data it could be argued that capsaicin is simply a non-specific membrane fluidizer similar to alcohols, fatty acids or other fluidizing agents. However, we were also able to demonstrate that capsaicin was unable to fluidize artificial liposomes constructed to provide a membrane with physical properties similar to those found in platelets. These membranes could be easily fluidized with either alcohols or A_2C suggesting that capsaicin produced its effect by other mechanisms. In support of this hypothesis we determined that the fluidizing effect of capsaicin was cell specific. Over the concentration range employed, capsaicin was able to fluidize plasma membranes of platelets, peritoneal mast cells and red blood cells but not enterocyte apical brush border membranes or the plasma membranes of B-lymphocytes. These observations were unlikely to have been secondary to differences in the capsaicin/lipid molar ratios. The highest such ratio was calculated for the microvillus membrane samples (1.43 at 40 μM) which produced no alteration in membrane physical properties. This was similar for the liposome samples that had the lowest capsaicin/lipid ratio of 0.14. However, erythrocytes that responded dramatically to the same concentration of capsaicin had capsaicin: lipid ratios intermediate between these two extremes of 0.33. Furthermore, the effect of capsaicin on plasma membrane physical properties was not limited to fluidization. At high concentrations capsaicin significantly reduced the fluidity of red cell membranes and a similar trend was apparent in peritoneal mast cells. These results could not be explained by the hypothesis that capsaicin was a simple membrane fluidizing agent.

In order to further investigate these effects we carefully dissected these responses in the red cell. Using intact cells a clear biphasic action of capsaicin could be elicited; at low doses significant fluidization was observed but, with increasing capsaicin concentrations the plasma membrane became less fluid. By preparing red cell ghosts and testing the activity capsaicin on the isolated membrane fraction we could demonstrate that the decrease in fluidity with high capsaicin concentrations was dependent upon the intact cell preparation. Furthermore, by extracting membrane lipids from the plasma membrane preparation and reconstituting liposomes it became apparent that capsaicin had no effect on isolated red cell plasma membrane lipids. Thus, our data would suggest that the fluidization induced by capsaicin is dependent upon a non-lipid extractable plasma membrane component. This effect can be observed in isolated membrane fragments and presumably does not require a cytoplasmic component. However, the decrease in fluidity with high concentrations of capsaicin is only evident in the whole cell preparation; presumably requiring both a plasma membrane site and a cytoplasmic component.

In summary, capsaicin has been touted as a pharmacological agent with specificity for sensory afferent neurons. While it undoubtedly has an action at this site it clearly also interacts with other cell types making an interpretation of its physiological effect in all but an isolated cell system difficult to establish. The effects of capsaicin on non-neuronal cells range from the functional effect of impairing platelet aggregation to cell specific interference with the maintenance of plasma membrane fluidity. Since the latter has been implicated in the regulation of many cell membrane events from ion and nutrient permeability to transporter function [29–32] it is likely that the effects of capsaicin will be extremely heterogenous.

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