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Capsaicin receptors mediate free radical-induced activation of cardiac afferent endings

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Abstract

Objective: The effects of capsaicin on sensory neurons are mediated by its interaction with a specific membrane receptor and opening of a non-selective cation channel. In the rat heart, capsaicin-sensitive nerve endings are known to be activated by oxygen radicals. We investigated the possibility that free oxygen radicals stimulate sensory nerve endings by acting upon the capsaicin receptor. **Methods:** We studied the effects of capsaicin (0.16–16.0 nmol), bradykinin (0.1–10 nmol), H_2O_2 (1.5–30 μmol), and xanthine + xanthine oxidase (X + XO, 1 μmol + 0.03 mU) applied to the surface of the rat heart for 30 s on the activity of cardiac, capsaicin-sensitive, vagal and sympathetic afferent fibers before and after blockade of capsaicin receptors with capsazepine (200 μg/kg, i.v.), a specific antagonist for the capsaicin receptor. **Results:** Application of capsaicin (0.32–16.0 nmol), H_2O_2 (9–30 μmol), bradykinin (1–10 nmol), and X + XO increased cardiac vagal and sympathetic afferent activity. Administration of capsazepine had no effect on the baseline activity of either vagal or sympathetic cardiac afferents, but it abolished the response of the afferent fibers to all doses of capsaicin, H_2O_2, and X + XO tested. Capsazepine had no effect on afferent activation by bradykinin. Administration of another capsaicin receptor blocker, ruthenium red (780 μg/kg, i.v.), had similar effects. **Conclusions:** The results of these experiments indicate that blockade of capsaicin receptors inhibits activation of vagal and sympathetic cardiac afferent fibers by free oxygen radicals. The fact that capsazepine and ruthenium red did not affect the afferent response to bradykinin suggests that this effect of the blockers was specific for capsaicin receptors. The possible functional implications of this interaction are discussed.

Keywords: Cardiac afferent; Capsaicin; Free radical; Ventricular receptor; Rat; Capsazepine

1. Introduction

Capsaicin, the pungent ingredient of *Capsicum* pepper, is a specific activator of nociceptive sensory neurons with C and Aδ fibers [1]. The effects of capsaicin on sensory neurons are mediated by its interaction with a specific membrane receptor and opening of a non-selective cation channel [2]. A specific antagonist of the capsaicin receptor, capsazepine, was recently developed [3], and its effectiveness in antagonizing the various physiological effects of capsaicin has been demonstrated [4–6]. Another capsaicin antagonist, ruthenium red, was described as a 'functional capsaicin antagonist' [7], because it does not interfere with the capsaicin-binding site of the receptor [8], but blocks the capsaicin-activated ion channel [9,10]. However, the effect of ruthenium red is selective since it attenuated capsaicin induced activation of nociceptors and release of sensory neuropeptides, but did not affect sensory neuron activation by other stimuli [10–12].

In previous experiments, we have demonstrated that capsaicin activates cardiac chemosensitive neurons with C-fiber vagal afferents [13,14]. These capsaicin-sensitive afferents could be activated by free oxygen radicals derived from hydrogen peroxide or xanthine/xanthine oxidase mixture [13] or produced endogenously at reperfusion after prolonged ischemia [14]. Interestingly, free oxygen radicals stimulated only those vagal afferent fibers that were capsaicin-sensitive. Similarly, experiments performed by Huang et al. [15,16] demonstrated that free oxygen radicals stimulate cardiac chemosensitive nerve endings

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with sympathetic C-fiber afferents. These endings also are known to be capsaicin-sensitive. These data prompted us to hypothesize that free oxygen radicals stimulate sensory nerve endings by acting through the capsaicin receptor.

In the present study we investigated this possibility by documenting the effects of capsaicin and free oxygen radicals on cardiac capsaicin-sensitive vagal and sympathetic afferent fibers before and after blockade of capsaicin receptors with either capsazepine or ruthenium red. We also documented the effect of bradykinin on the afferent endings to assess the possibility that the capsaicin antagonists could have altered afferent responses to a mediator known to act independently of the capsaicin receptor [17].

2. Methods

Sprague-Dawley rats (250–320 g, males) were anesthetized intraperitoneally with a mixture of 2% α-chloralose and 25% urethane in saline (3 ml/kg). The lungs were ventilated by a rat respirator (60 breaths/min, Harvard Apparatus, South Natick, MA) with air, supplemented with O_2 (50%). Polyethylene catheters were inserted in a carotid artery and jugular vein for measurement of arterial pressure and administration of drugs, respectively. Arterial and tracheal pressures were measured by strain gauges (model 1270A, Hewlett Packard, Andover, MA), and heart rate was measured by a cardiotachometer (Coulburn Instruments, Allentown, PA) triggered by the arterial pressure pulse. Heart rate, arterial pressure and nerve discharge (see below) were recorded by a thermal recorder (model MT95000, Astro-Med, West Warwick, RI).

Vagal afferent impulses were recorded from fine slips of the left cervical vagus. Sympathetic afferent impulses were recorded from fine slips of the left stellate nerve dissected from the stellate ganglion. Nerves were covered with mineral oil and placed on a silver electrode. Impulses were amplified (model P511, Grass Instruments, Quincy, MA), displayed on an oscilloscope (model 450, Gould Instrument Systems, Valley View, OH), and fed into a rate meter (Frederick Haer, Brunswick, ME) whose window discriminators were set to accept potentials of a particular amplitude. Impulses were counted by ratemeter in 1-s bins. Bundles that had one, or at most two, easily distinguishable active fibers were used. We studied only spontaneously active fibers which had receptive fields in the heart that could be located precisely.

Chemosensitivity of an ending was tested by intravenous injection and epicardial application of capsaicin to the surface of the heart. Capsaicin (1 mg/ml) was dissolved in saline containing 10% ethanol and 1% Tween-80 and then diluted to the final concentrations with saline.

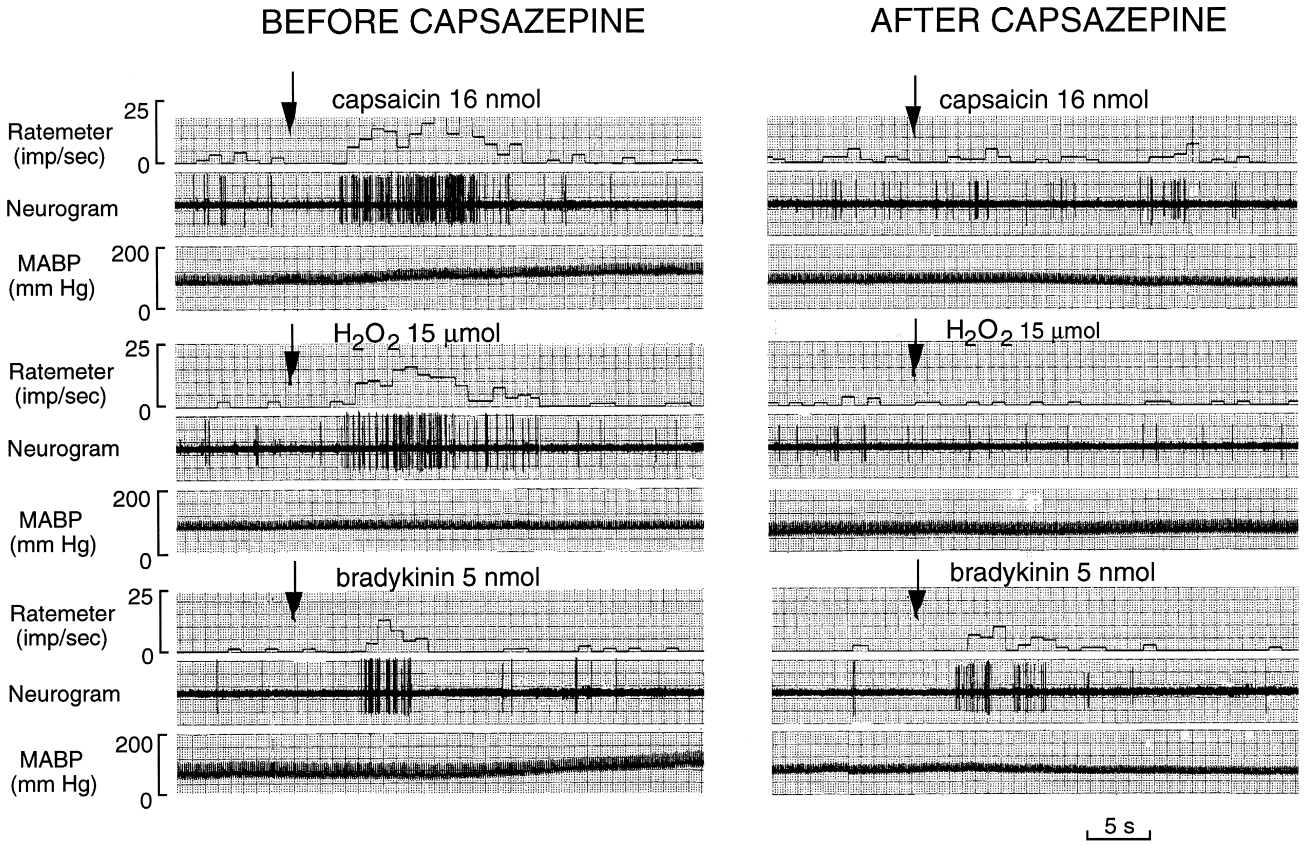

Fig. 1. Representative tracings of the changes in activity of cardiac vagal chemosensitive fiber with the receptive field in the left ventricle in response to epicardial application of capsaicin (16 nmol), H_2O_2 (15 μmol), and bradykinin (5 nmol) to the receptive field before and after administration of capsazepine (200 μg/kg, i.v.). Arrow indicates the moment of drug application. MABP, mean arterial blood pressure; imp/s, impulses per second.

Capsaicin was injected intravenously in doses of 1.6–3.2 nmol (0.1–0.2 ml of 50 μg/ml solution) and then, if the fiber was activated, capsaicin (0.16–16 nmol in 10 μl) was applied directly to surface of the heart with a small circle of filter paper 3 mm in diameter to determine the receptive field. We considered the fiber to be activated when its activity increased by more than 25% above the baseline. Once the receptive field was localized, bradykinin (0.1–10 nmol in 10 μl of saline), H_2O_2 (1.5–30 μmol in 10 μl of saline), and X + XO mixture (1 μmol and 0.03 mU respectively in 10 μl of saline) were applied to the epicardial surface encompassing the receptive field with filter paper for 30 s. A period of 30 s is sufficient for the afferent endings to achieve a maximum response to the chemical application [13]. All chemicals were tested in random order. Applications of capsaicin vehicle or saline alone were tested and found to have no effect on fiber activity.

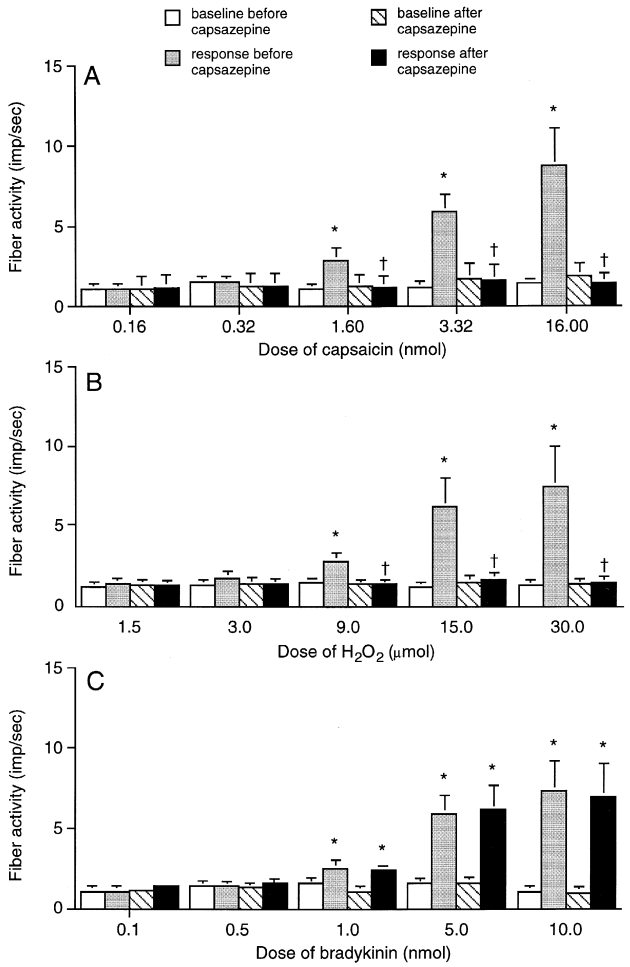

Fig. 2. Effect of capsazepine (200 μg/kg, i.v.) on the dose-related activation of cardiac chemosensitive endings with vagal afferent fibers in response to epicardial application of capsaicin (panel A), H_2O_2 (panel B), and bradykinin (panel C). Open bars = baseline before capsazepine administration; gray bars = response to chemical agonist before capsazepine administration; hatched bars = baseline after capsazepine administration; black bars = response to chemical agonists after capsazepine administration. * $P < 0.05$ response vs. respective baseline, † $P < 0.05$ response before vs. response after capsazepine administration. imp/s, impulses per second.

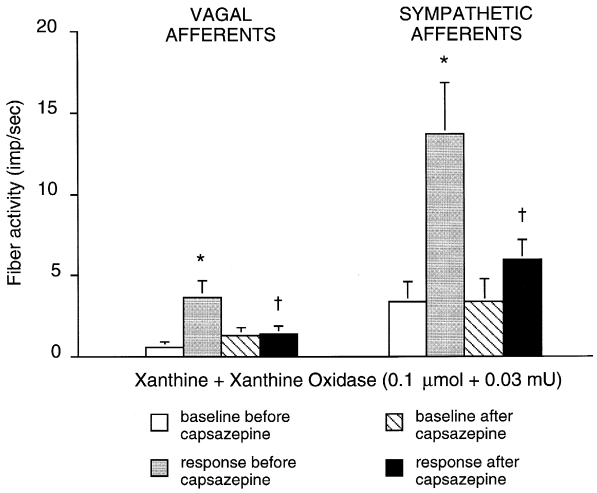

Fig. 3. Effect of capsazepine (200 μg/kg, i.v.) on activation of cardiac chemosensitive vagal and sympathetic afferent endings in response to epicardial application of xanthine + xanthine oxidase mixture (1 μmol + 0.03 mU). Legend, symbols, and abbreviations as in Fig. 2.

After each application, the paper was removed and the surface of the heart was washed with warm saline. Intervals of 10–15 min were allowed between applications. If the fiber was activated with capsaicin, bradykinin, H_2O_2, and/or xanthine/xanthine oxidase, applications of the effective doses were repeated 5 min after intravenous administration of either capsazepine (200 μg/kg, i.v.) or ruthenium red (780 μg/kg, i.v.). In control experiments, applications of effective doses of the drugs were repeated after administration of capsazepine or ruthenium red vehicle to assure that the response was reproducible.

Capsazepine (5 mg) was dissolved in 0.133 ml of DMSO and then diluted with saline containing 10% of ethanol and 1% of Tween-80 to a concentration of 200 μg/ml. Ruthenium red was prepared at the concentration 780 μg/ml in saline. Administration of capsazepine vehicle or saline had no effect on fiber responses to chemical agonists. Capsazepine was obtained from Research Biochemicals International (Natick, MA); all other drugs were obtained from Sigma Chemical (St. Louis, MO).

Experiments performed by Franco-Cereceda and coauthors [18,19] in the isolated perfused guinea-pig heart, showed that capsazepine inhibited the release of calcitonin gene-related peptide from cardiac sensory nerves caused by a decrease in pH of the perfusion solution. They suggested that acidosis may cause the release of some endogenous ligand for the capsaicin receptor. We considered the possibility that the pH of our H_2O_2 solution and the X + XO mixture was responsible for activation of the afferents, and thus, the effect of capsazepine was due to its ability to inhibit pH-induced activation of the sensory endings. Therefore, in additional experiments with vagal and sympathetic afferent fibers ($n = 5$ in both), we studied the effect of capsazepine on afferent responses to applica-

Table 1
Impulse activity (imp/s) from cardiac vagal chemosensitive afferent fibers in response to epicardial applications of capsaicin, H_2O_2, X+XO, and bradykinin before and after administration of capsazepine vehicle

Agonist	Before vehicle administration (imp/s)		After vehicle administration (imp/s)	
	Baseline	Response	Baseline	Response
Capsaicin 3.2 nmol ($n=10$)	1.2±0.2	5.9±1.1*	1.3±0.3	6.1±1.5*
Bradykinin 5.0 nmol ($n=10$)	1.1±0.3	7.3±1.9*	1.3±0.2	6.9±2.0*
H_2O_2 15 μmol ($n=10$)	1.2±0.2	6.2±1.8*	1.2±0.2	5.8±1.3*
X±XO 1 μmol±0.03 mU ($n=5$)	0.6±0.3	3.4±1.3*	0.8±0.3	4.3±1.1*

* $P<0.05$ response vs. respective baseline.

tions of 10 μl of HCl solutions with pH in the range of 3.0–7.0.

We also considered the possibility that capsazepine and ruthenium red possess free radical scavenging properties that can explain their inhibitory effect on H_2O_2 and X+XO-induced responses of cardiac afferents. For this reason, we tested the ability of capsazepine and ruthenium red to inhibit the reduction of nitroblue tetrazolium (NBT) mediated by superoxide anion in non-enzymatic (phenazine methosulfate–NADH) reactions in vitro and compared it with free radical scavenging properties of superoxide dismutase (SOD) [20]. The reaction mixture contained 1 mM of NBT, 30 μM of phenazine methosulfate and 156 μM of NADH in 0.1 M phosphate buffer, pH 7.4. Reduction of NBT was measured by a Milton-Roy spectrophotometer as a change in absorbency at 560 nm during 5 min either in the presence or in the absence of increasing concentrations of capsazepine, ruthenium red, or SOD.

Reported values are means ± s.e.m. The firing rate of afferent fibers was calculated as the average number of impulses per second (imp/s) over a period of 10 s of maximal activity during 60 s immediately before and during the 60 s after drug application. Threshold doses of capsaicin, H_2O_2, and bradykinin necessary to activate the endings were calculated from the dose–response curves using least-squares regression analysis. Differences among treatments were determined by analysis of variance for repeated measures, and differences between means were isolated by the Bonferroni correction for multiple *t*-tests. Statistical significance was accepted at $P<0.05$.

These investigative procedures conform with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

3. Results

3.1. Effect of capsazepine and ruthenium red on the responses of cardiac vagal afferent fibers to chemical agonists

In 26 rats we obtained recordings from a total of 41 vagal afferent fibers with receptive fields in various areas of the heart. Twenty four of these endings were capsaicin sensitive (1.6–16.0 nmol). Each of the capsaicin-sensitive fibers was also activated by epicardial application of bradykinin (1.0–10.0 nmol). Endings that were not cap-

Table 2
Effect of ruthenium red (780 μg/kg, i.v.) on impulse activity (imp/s) from cardiac chemosensitive endings with vagal afferent fibers in response to epicardial applications of capsaicin, H_2O_2, X+XO, and bradykinin

Dose of agonist	Before ruthenium red (imp/s)		After ruthenium red (imp/s)	
	Baseline	Response	Baseline	Response
Capsaicin				
3.2 nmol ($n=6$)	0.4±0.2	3.9±1.6*	0.4±0.2	0.5±0.3†
32 nmol ($n=6$)	0.7±0.3	9.1±1.8*	0.3±0.2	0.5±0.3†
H_2O_2				
9.0 μmol ($n=6$)	0.7±0.3	2.2±0.4*	0.4±0.2	0.4±0.3†
15 μmol ($n=5$)	0.8±0.3	7.5±2.1*	0.5±0.2	0.5±0.3†
X+XO ($n=6$)				
1 μmol+0.03 mU	1.1±0.2	6.5±0.8*	0.8±0.3	1.3±0.5†
Bradykinin				
1 nmol ($n=5$)	1.1±0.2	2.5±0.4*	0.8±0.3	3.5±1.4*
10 nmol ($n=5$)	0.9±0.3	7.4±1.7*	0.7±0.3	7.8±1.2*

* $P<0.05$ response vs. respective baseline.
† $P<0.05$ response before vs. response after ruthenium red administration.

saicin-sensitive did not respond to applications of bradykinin. Seventeen of the 24 capsaicin sensitive endings responded to epicardial application of H_2O_2 (9.0–30.0 μmol) as well. Only the endings that responded to all three agonists (capsaicin, H_2O_2, and bradykinin) were used in this study. These chemosensitive endings did not exhibit characteristics of mechanosensitive endings, such as a cardiac or respiratory rhythm or a responsiveness to changes in cardiac hemodynamics. The receptive fields of these fibers were located: 5 in the right atrium, 3 in the left atrium and 9 in the left ventricle. The average maximum baseline activity for these vagal afferent fibers was 1.1 ± 0.2 imp/s. Epicardial capsaicin application evoked an increase in fiber activity with a latency of 4–6 s; the threshold dose of capsaicin was 0.54 ± 0.23 nmol as determined by regression analysis. H_2O_2 increased the firing rate of the vagal fibers with a latency of 2–6 s at a threshold dose of 3.47 ± 0.81 μmol. Application of bradykinin stimulated vagal afferent endings at a threshold dose of 0.65 ± 0.3 nmol with a latency of 4–8 s. Fig. 1 illustrates the response of a representative vagal afferent fiber from the left ventricle to epicardial application of capsaicin, H_2O_2, and bradykinin (left panels). The dose–response effects of epicardial applications of capsaicin (A), H_2O_2 (B), and bradykinin (C) on cardiac vagal afferent activity are illustrated in Fig. 2.

Administration of capsazepine had no effect on the baseline activity of the vagal endings which averaged 1.1 ± 0.2 before and 1.1 ± 0.3 imp/s 5 min after capsazepine administration. Capsazepine completely abolished the response of vagal afferent fibers to capsaicin and H_2O_2 and had no effect on the afferent responses to bradykinin (Fig. 1, Fig. 2). Five cardiac vagal endings were tested with epicardial applications of the xanthine/xanthine oxidase mixture; activity of their afferent fibers in response to xanthine/xanthine oxidase increased from 0.6 ± 0.3 to 3.4 ± 1.3 imp/s ($P < 0.01$) The afferent response to xanthine/xanthine oxidase also was abolished by capsazepine (Fig. 3). Applications of capsaicin, bradykinin, H_2O_2 or X + XO after administration of capsazepine vehicle induced afferent responses that did not differ from responses at the same doses before vehicle administration (Table 1).

Table 2 presents the data on the effect of ruthenium red on the responses of cardiac vagal chemosensitive afferents to epicardial applications of chemicals agonists. Predictably, administration of this capsaicin antagonist inhib-

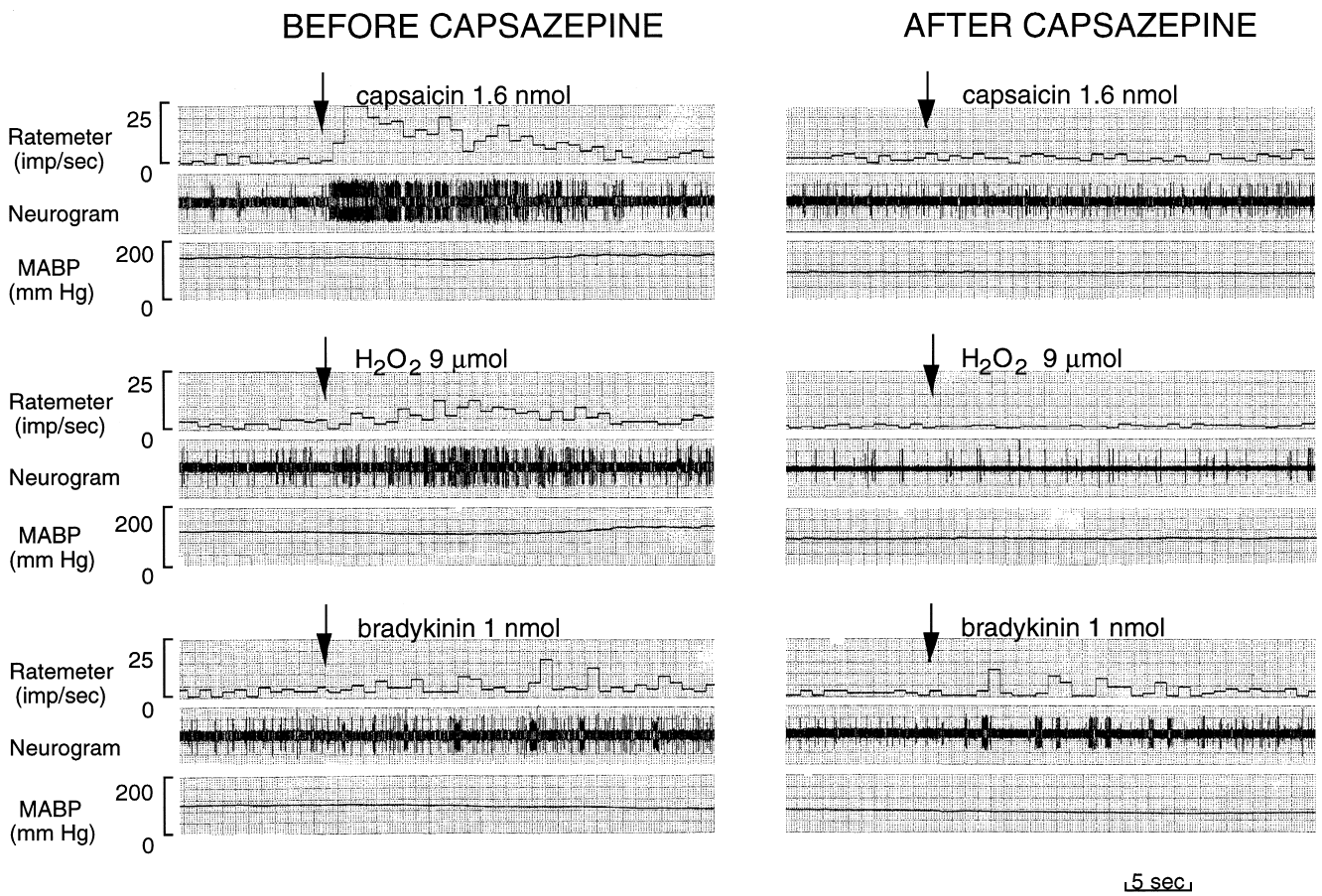

Fig. 4. Representative tracings of the changes in activity of a cardiac sympathetic afferent fiber with the receptive field in the left ventricle in response to epicardial application of capsaicin (1.6 nmol), H_2O_2 (9 μmol) and bradykinin (1 nmol) to the receptive field before and after administration of capsazepine (200 μg/kg, i.v.). Arrow indicates the moment of drug application. Abbreviations as in Fig. 1.

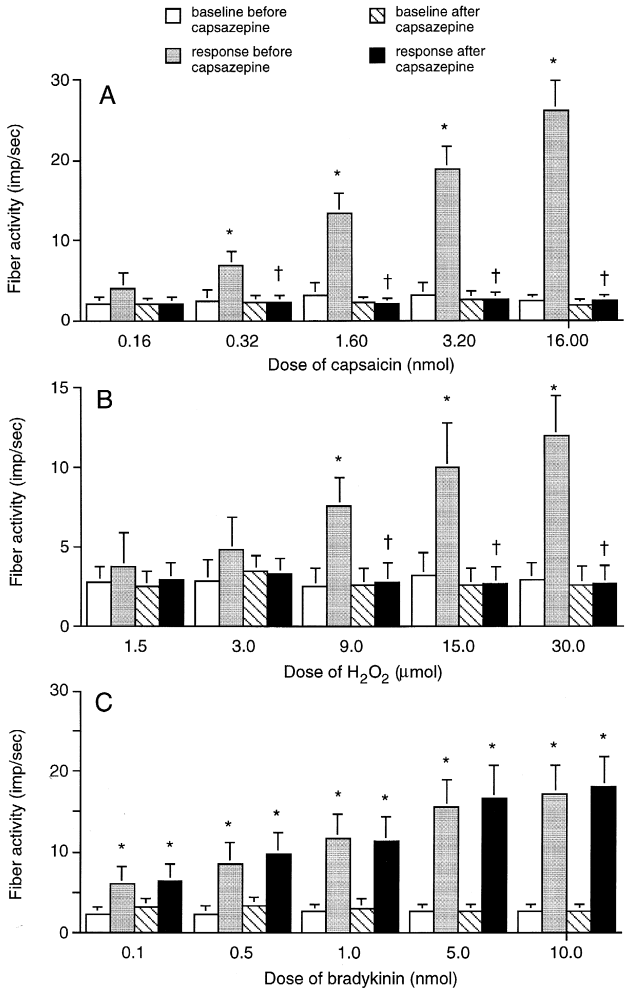

Fig. 5. Effect of capsazepine (200 μg/kg, i.v.) on the dose-related activation of cardiac chemosensitive endings with sympathetic afferent fibers in response to epicardial application of capsaicin (panel A), H_2O_2 (panel B), and bradykinin (panel C) to their receptive field. Legend, symbols, and abbreviations as in Fig. 2.

ited activation of vagal afferent fibers by capsaicin. Responses to epicardial applications of H_2O_2 and X + XO were also completely abolished after administration of ruthenium red. No significant effects on the responses of vagal afferents to bradykinin were observed after administration of this inhibitor.

3.2. Effect of capsazepine on the responses of cardiac sympathetic afferent fibers to chemical agonists

We obtained recordings from a total of 19 cardiac sympathetic afferent fibers. The receptive fields of these fibers were located on the anterior surface of the left ventricle. All of these endings responded to epicardial applications of capsaicin (0.16–16.0 nmol), H_2O_2 (3.0–30.0 μmol) and bradykinin (0.1–10.0 nmol). Fig. 4 illustrates the response of a representative sympathetic afferent fiber to epicardial application of capsaicin, H_2O_2, and bradykinin (left panels). Sensitivity to each of these chemical stimuli was markedly greater for the sympathetic afferent fibers than for vagal afferents. The sympathetic afferent fibers responded to a threshold dose of capsaicin of 0.10 ± 0.08 nmol with a latency of 1 s or less. H_2O_2 increased the firing rate of these fibers with a latency of 1–2 s at a threshold dose of 1.51 ± 0.22 μmol. The threshold dose for bradykinin was 0.05 ± 0.02 nmol with a similar short latency. The higher sensitivity and shorter latency of response of sympathetic afferents to epicardial application of these drugs may be due to the more superficial location of the endings in the myocardium as compared to the vagal afferents [21]. Nine sympathetic afferent fibers were tested with epicardial applications of xanthine/xanthine oxidase. All these fibers were sensitive to xanthine/xanthine oxidase, and their discharge increased from 3.3 ± 1.2 to 13.7 ± 3.0 imp/s ($P < 0.01$) (Fig. 3).

Fig. 5 illustrates dose–response effect of the sympathetic cardiac fibers to epicardial applications of capsaicin (A), H_2O_2 (B), and bradykinin (C) before and after administration of capsazepine. The responses of sympathetic afferent fibers to capsaicin and H_2O_2 were abolished after capsazepine administration (Fig. 4, Fig. 5A,B). The response of the sympathetic afferents to xanthine/xanthine oxidase after capsazepine administration also was significantly inhibited (Fig. 3). Capsazepine had no effect on the response of cardiac sympathetic afferents to bradykinin (Fig. 4, Fig. 5C).

Administration of ruthenium red inhibited activation of sympathetic afferent fibers by capsaicin. Responses to epicardial applications of H_2O_2 and X + XO were also completely abolished after administration of ruthenium

Table 3
Effect of capsazepine (200 μg/kg, i.v.) on impulse activity (imp/s) from cardiac vagal and sympathetic chemosensitive afferent fibers in response to epicardial applications of HCl solutions

pH	Vagal afferents (imp/s)				Sympathetic afferents (imp/s)			
	Before capsazepine		After capsazepine		Before capsazepine		After capsazepine	
	Baseline	Response	Baseline	Response	Baseline	Response	Baseline	Response
7.0	1.2±0.2	0.9±0.1	1.2±0.3	1.3±0.3	1.9±0.4	2.1±0.5	1.9±0.3	1.9±0.4
6.0	1.1±0.3	1.3±0.2	1.2±0.3	1.5±0.4	2.2±0.6	1.9±0.5	2.1±0.3	2.0±0.6
5.0	1.1±0.2	1.2±1.8	1.1±0.4	1.5±0.4	2.2±0.6	1.8±0.4	2.0±0.4	2.3±0.5
4.0	0.9±0.2	4.0±1.4*	1.1±0.3	1.4±0.6	2.3±0.5	8.7±2.4*	2.3±0.6	2.6±0.5
3.0	1.1±0.2	6.4±1.3*	1.3±0.3	1.3±0.4	1.8±0.4	9.3±2.1*	2.3±0.4	2.7±0.5

* $P < 0.05$ response vs. respective baseline.

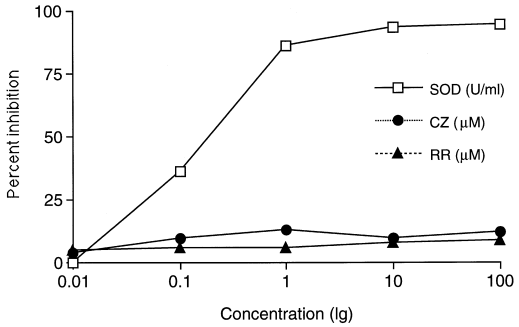

Fig. 6. Effect of superoxide dismutase (SOD), capsazepine (CZ), and ruthenium red (RR) on the reduction of nitroblue tetrazolium (NBT) mediated by superoxide anion in the non-enzymatic (phenazine methosulfate–NADH) reaction. The reaction mixture contained 1 mM of NBT, 30 μM of phenazine methosulfate and 156 μM of NADH in 0.1 M phosphate buffer, pH 7.4. Abscissa: concentration of SOD, capsazepine, or ruthenium red (log). Ordinate: percent inhibition of NBT reaction.

red. No significant effects on the responses of the sympathetic afferents to bradykinin were observed after administration of this inhibitor.

3.3. Effect of capsazepine on the responses of vagal and sympathetic afferent fibers to epicardial HCl

Table 3 summarizes the response of cardiac vagal and sympathetic afferents fibers to epicardial applications of HCl solutions with a pH from 3.0 to 7.0. Stimulation of vagal afferent fibers ($n = 5$) occurred only with pH 4.0 or lower. Similarly, none of the tested sympathetic fivers ($n = 5$) were activated by a pH above 4.0. Capsazepine abolished activation of vagal and sympathetic afferent fibers caused by the HCl solutions with pH 3.0–4.0.

3.4. Free radical scavenging properties of capsazepine and ruthenium red

Fig. 6 illustrates the ability of capsazepine, ruthenium red, or SOD to inhibit NBT reduction mediated by the production of superoxide anion. Results are expressed as percent inhibition of NBT reaction in control conditions, without capsazepine, ruthenium red or SOD. In control conditions the increase in absorbency at 560 nm was 0.025 OD/min. SOD inhibited the reaction with an IC_{50} of 2.9 U/ml. Capsazepine and ruthenium red in the dose range of 0.01–100 μM had no effect on the NBT reaction suggesting that these drugs do not possess free radical scavenging activity.

4. Discussion

The results of these experiments provide evidence that blockade of capsaicin receptors with either capsazepine or ruthenium red inhibits activation of both vagal and sympathetic cardiac afferent fibers by free oxygen radicals. The fact that these agents did not affect the afferent response to bradykinin suggests that this effect of the blockers was specific for capsaicin receptors [17].

Recently, Franco-Cereceda and co-authors [18,19] showed in the isolated perfused guinea-pig heart that a decrease in pH of the perfusion solution caused the release of calcitonin gene-related peptide from the sensory nerves; capsazepine inhibited this effect. They suggested that acidosis may cause the release of some endogenous ligand for the capsaicin receptor. We considered the possibility that the pH of our H_2O_2 solution and a X + XO mixture was responsible for activation of the afferents when these chemicals were applied; and thus, the effect of capsazepine to inhibit these responses was due to its ability to inhibit pH-induced activation of the sensory endings rather than free-radical induced activation. The pH of 30 μM H_2O_2 solution (the highest concentration we used for epicardial applications) is 5.8, and that of X + XO mixture is 7.2. In our experiments we found that epicardial applications of 10 μl of HCl solutions with pH in the range of 5.0–7.0 did not activate vagal or sympathetic afferent fibers. These findings are in accordance with the data of Uchida and Murao [22] which showed that the threshold pH for activation of cardiac sympathetic fibers with epicardial application of HCl was 3.85–3.20. Furthermore, we have demonstrated previously that antioxidants completely prevent activation of the cardiac vagal afferent fibers in response to either H_2O_2 or X + XO, suggesting that this activation was indeed mediated by oxygen free radicals [13]. Thus, it is not likely that changes in pH mediated the effects of oxygen free radicals in our study.

We also considered the possibility that capsazepine and ruthenium red possess free radical scavenging properties that can explain their inhibitory effect on H_2O_2 and X + XO-induced responses of cardiac afferents. We found that capsazepine and ruthenium red have no effect on the reduction of NBT mediated by formation of superoxide anion in vitro. We concluded that observed effects of capsazepine and ruthenium red could not be explained by their free radical scavenging activity, but were due to its capsaicin receptor inhibiting properties.

We and other studies have shown that free oxygen radicals can stimulate chemosensitive sensory nerve endings in the heart [13–15] producing reflex responses mediated by these afferents [16], but the mechanism of their effect on afferent endings remains uncertain. It is possible that oxygen radicals may cause the release of an yet unknown endogenous ligand for the capsaicin receptor, similar to that proposed by Franco-Cereceda et al. [18]. Alternatively, it is known that free oxygen radicals are able to change properties of membrane-bound integral proteins [23,24], probably by affecting the protein tertiary structure and membrane lipid viscosity. It is possible that the capsaicin receptor is the one of the proteins that is modulated by free oxygen radicals thus mediating their action on afferent fibers.

The capsaicin receptor comprises a non-selective cationic channel which evokes an inward depolarizing current upon binding with the agonist. One can postulate that free oxygen radicals cause a conformational change of the integral protein of the receptor and thus increase permeability of the cationic channel. Binding of the receptor with specific inhibitor, capsazepine, prevents this effect probably by stabilization of its tertiary structure. The molecular mechanism of the free oxygen radical interaction with the capsaicin receptor requires further investigation.

The functional role of the capsaicin receptor has remained a mystery since its discovery [25]. Our results suggest a possible role for the capsaicin receptor in mediating cardiac reflex responses to oxygen radicals. Recently we demonstrated that reflex changes in renal sympathetic nerve activity after prolonged ischemia of the rat heart and during reperfusion are mediated by activation of vagal and sympathetic afferent endings by oxygen-derived free radicals and can be abolished with the antioxidant, desferoxamine [26]. In the light of these results it is possible that the capsaicin receptor may play an important role in the disturbances of autonomic regulation during ischemia and reperfusion and in other numerous pathological states associated with the increased formation of oxygen-derived free radicals.

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