Electrophysiological effects of capsaicin on human atrial fibers

LI Qian[1], LIU Su[2], CHEN Zi-Ying[2], LIU Yi-Xian[1], ZHANG Wen-Li[2], ZHAO Hong[2], LIU Lin-Li[2], HE Rui-Rong[1*]

(1. *Department of Physiology, Institute of Basic Medicine, Hebei Medical University, Shijiazhuang* 050017, *China*; 2. *Department of Cardiac Surgery, the Second Affiliated Hospital of Hebei Medical University, Shijiazhuang* 050000, *China*)

Abstract: **AIM** To study the electrophysiological effects of capsaicin on human atrial fibers. **METHODS** Parameters of action potential in human atrial specialized fibers were recorded using standard intracellular microelectrode technique. **RESULTS** Capsaicin (1 – 30 $\mu mol \cdot L^{-1}$) decreased the amplitude of action potential, maximal rate of depolarization, velocity of diastolic (phase 4) depolarization and rate of pacemaker firing, and shortened the 90% action potential duration in a concentration-dependent manner. L-type Ca^{2+} channel agonist Bay K8644 (0.5 $\mu mol \cdot L^{-1}$) antagonized the inhibitory effects of capsaicin on human atrial fibers. Pretreatment of the fibers with capsazepine (10 $\mu mol \cdot L^{-1}$), a competitive capsaicin antagonist, failed to influence the electrophysiological effects of capsaicin. **CONCLUSION** Capsaicin exerted a negative chronotropic action and accelerated the repolarization of human atrial specialized fibers which may be due to reduction in calcium influx and not mediated by capsaicin receptors.

Key words: capsaicin; heart atrium; electrophysiology; calcium

CLC number: R972
Document code: A
Article ID: 1000-3002(2005)01-0013-05

Capsaicin, the main pungent component of chili peppers, has long been known to influence cardiovascular functions. These actions on heart are mediated by its interaction with a specific vanilloid receptor 1(VR_1) on sensory nerve endings in cardiac muscles, including the liberation of neuropeptides from the vanilloid-sensitive innervation of the heart[1,2]. Capsaicin also inhibited K^+ and Na^+ currents in cardiac cells[3,4]. Recently, we have found that capsaicin shortened the duration of action potential in normal guinea pig papillary muscles and also decreased maximal velocity of phase 0 depolarization in partially depolarized papillary muscles[5]. Furthermore, capsaicin also exerted a negative chronotropic action and induced a delayed repolarization of pacemaker cells in sinoatrial node and atrioventricular node of rabbits[6,7]. However, the effects of capsaicin on human atrial fibers have not yet been elucidated so far. It is established that there are two types of fibers in human atrium: the first shows electrical characteristics typical of atrial contractile cells and the second shows those of atrial specialized fibers. Automaticity developed only in the latter type of cells[8,9]. The present study was undertaken to investigate the electrophysiological effects of capsaicin on human atrial specialized fibers and its action mechanisms.

1 MATERIALS AND METHODS

1.1 Preparation

Small pieces ($< 1\ cm^2$) of atrial myocardium from the anterior free wall of the right atrium were excised from the hearts of 18 patients undergoing corrective open heart surgery as part of the cannulation technique for cardiopulmonary bypass. Prior to surgery, approvement of hospital ethics committee and informed consent were obtained. All patients were under 16 years old and suffered from

Received date: 2004-06-08 **Accepted date**: 2004-10-15

Biographies: LI Qian (1977 –), female, native of Shijiazhuang, Hebei Province, master degree, major research field is cardiovascular physiology; HE Rui-Rong (1928 –), male, native of Wuxi, Jiangsu Province, professor, major research field is cardiovascular and neural physiology.

* Corresponding author. E-mail: syho @ hebmu. edu. cn
Tel and Fax: 86-311-6062490

congenital heart diseases including ventricular septal defects, 13; atrial septal defects, 3; and tetralogy of Fallot, 2. To ensure that the preparations of atrial tissue were physiologically normal, the following criteria were employed. No patient had been in congestive heart failure and none had received any cardiotonic, antiarrhythmic, or diuretic medication. Preoperative electrocardiograms in all patients revealed normal values for P-R interval, P-wave amplitude, and P-wave duration. No patient had a history or electrocardiographic evidence of cardiac arrhythmia.

Immediately after excision, the tissue was immersed in cold Tyrode's solution and taken to the laboratory. Then the preparation was fixed with fine pins to the silica gel on the base of a perfusion chamber and equilibrated for 1 h. The preparation was superfused (4 mL·min^{-1}) with Tyrode's solution (35.5 – 36.5℃) of the following composition (mmol·L^{-1}): NaCl 137, $NaHCO_3$ 12, NaH_2PO_4 1.8, $MgCl_2$ 0.5, $CaCl_2$ 2.7, KCl 4, and dextrose 5.5. The Tyrode's solution was saturated by a mixture of 95% O_2 and 5% CO_2 and the pH was 7.30 – 7.40.

1.2 Electrophysiological measurements

Transmembrane action potentials were recorded from human atrial fibers with a glass microelectrode filled with 3 mol·L^{-1} KCl (a tip resistance of 10 – 20 MΩ), coupled to a high input impedance amplifier (MEZ 8201, Nihon Kohden). The amplified signals were fed to the A/D convertor and processed by a microcomputer. Maximal diastolic potential (MDP), amplitude of action potential (APA), maximal rate of depolarization (V_{max}), velocity of diastolic (phase 4) depolarization (VDD), rate of pacemaker firing (RPF) and 90% action potential duration (APD_{90}) were analyzed with the system of sampling and progressing cardiac transmembrane potential by microcomputer designed by our department[10]. Parameters were stored in the microcomputer for later analysis.

1.3 Experimental protocols

After 60 min of equilibration, the preparations were explored with glass microelectrodes to find those cells with spontaneous electric activity. Cells were accepted as atrial specialized fibers if their intracellular potentials showed the characteristics of "pacemaker" cells, a transition from slow depolarization of phase 4 to the more rapid depolarization of phase 0.

Action potentials (AP) were recorded after an equilibration time of 60 min. The experiments consisted of 3 groups: ① Effects of capsaicin on the electrophysiology of human atrial fibers. After recording of 3 control AP, capsaicin 1, 10 and 30 μmol·L^{-1} were applied in turn. AP were recorded at 1, 3, 5, 10, 15 min after application of each concentration, and capsaicin was washed off before the next dose. ② Effects of Bay K8644 on capsaicin-induced changes in AP of pacemaker cells. The effects of capsaicin (10 μmol·L^{-1}) alone were observed. Then after pretreatment with Bay K8644 (0.5 μmol·L^{-1}) for 10 min, capsaicin (10 μmol·L^{-1}) was added and AP were recorded at 15 min after application of capsaicin. ③ Effects of capsazepine on the action of capsaicin. The effects of capsaicin (10 μmol·L^{-1}) alone were observed. Then after pretreatment with capsazepine (10 μmol·L^{-1}) for 10 min, capsaicin (10 μmol·L^{-1}) was added and AP were recorded at 15 min after application of capsaicin. In each experiment, the preparation was washed with Tyrode's solution after application of drugs to observe the recovery of AP.

1.4 Drugs

Drugs used in this study included capsaicin, Bay K8644 and capsazepine (Sigma Chemical Co., USA). Capsaicin was dissolved in distilled water containing 10% ethanol and 1% Tween-80 and then diluted to final concentration with saline. Bay K8644 and capsazepine were prepared as stock solutions in alcohol and dimethyl sulfoxide (DMSO), respectively. Final concentration of alcohol and DMSO was 0.1% and 0.05%, respectively.

1.5 Statistical analysis

All data were presented as $\bar{x} \pm s$. Statistical differences were evaluated by paired t test.

2 RESULTS

2.1 Effects of capsaicin on transmembrane action potential

Compared with control group, capsaicin (1 – 30 μmol·L^{-1}) decreased APA, V_{max}, VDD, RPF and APD_{90} in a concentration-dependent manner (Tab 1, Fig 1). The changes in RPF induced by capsaicin paralleled to those of VDD. The above effects occurred after 3 min of superfusion of capsaicin and reached the peak within 5 – 10 min. The vehicle of capsaicin showed no effect on parameters of AP of atrial fibers

2.2 Effects of Bay K8644 or capsazepine on capsaicin-induced changes on action potentials

L-type calcium channel agonist Bay K8644 0.5 μmol·L^{-1} significantly increased APA, V_{max}, VDD and RPF. Pretreatment of the fibers with Bay K8644 abolished the effects of capsaicin 10 μmol·L^{-1}(Tab 2). The vehicle of Bay K8644 (0.1% alcohol in superfusate) had no effect on parameters of AP of atrial fibers.

Competitive capsaicin antagonist capsazepine 10 μmol·L^{-1} had no effect on AP. Pretreatment with capsazepine 10 μmol·L^{-1} failed to affect the above mentioned effects induced by capsaicin 10 μmol·L^{-1}(Tab 2). The vehicle of capsazepine (0.05% DMSO in superfusate) had no effect on parameters of AP of atrial fibers.

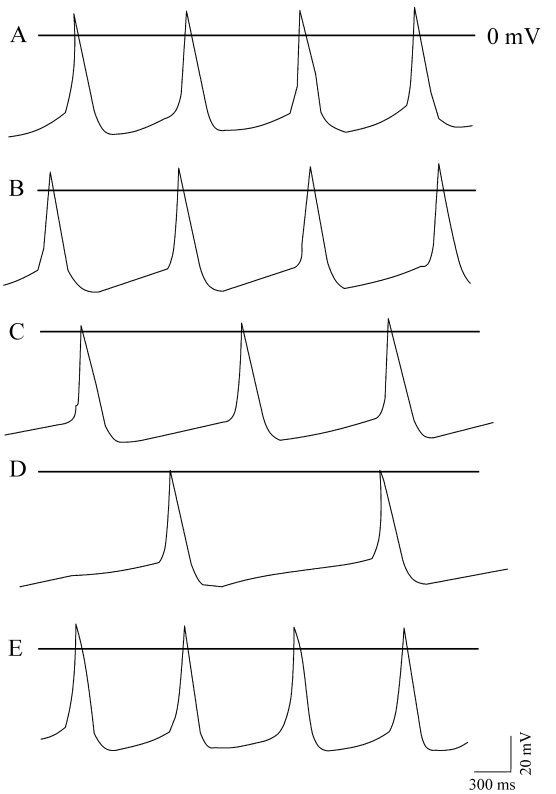

Fig 1. Original recording showing the effects of capsaicin on transmembrane action potentials of human atrial fiber. A: control; B, C, D: capsaicin 1, 10, 30 μmol·L^{-1}, respectively; E: wash out. The pictures of B, C and D were recorded at 15 min after application of capsaicin, respectively.

3 DISCUSSION

It has been widely accepted that calcium current plays an important role in pacemaker depolarization[11,12] and action potential upstroke of human atrial special fibers was generated to a great extent by I_{Ca}[9]. The present study showed that

Tab 1. Effects of capsaicin on transmembrane action potential of human atrial fibers

Capsaicin/μmol·L^{-1}	MDP/mV	APA/mV	V_{max}/V·s^{-1}	VDD/mV·s^{-1}	RPF/min^{-1}	APD_{90}/ms
0	(– 54.9 ± 6.1)	(67.6 ± 4.6)	(19.0 ± 2.3)	(16.0 ± 4.7)	(53.8 ± 10.0)	(270 ± 60)
1	– 1.5 ± 3.5	– 1.1 ± 1.5	0.1 ± 0.6	– 1.7 ± 3.2	– 5.7 ± 4.2*	0 ± 7
10	– 2.6 ± 4.3	– 6.9 ± 2.0**	– 4.4 ± 2.4**	– 6.1 ± 3.3**	– 20.1 ± 9.9**	– 18 ± 11*
30	– 0.9 ± 3.2	– 9.9 ± 3.2**	– 8.6 ± 3.4**	– 8.9 ± 4.4**	– 30.3 ± 11.8**	– 31 ± 16**

Capsaicin 1, 10 and 30 μmol·L^{-1} were applied in turn. AP were recorded at 15 min after application of each concentration, and capsaicin was washed off before the next dose. The results given are differences between control and a given group, and those bracketed in control group are original readings. MDP: maximal diastolic potential; APA: amplitude of action potential; V_{max}: maximal rate of depolarization; VDD: velocity of diastolic (phase 4) depolarization; RPF: rate of pacemaker firing; APD_{90}: 90% of duration of action potential. $\bar{x} \pm s$, $n = 6$. * $P < 0.05$, ** $P < 0.01$, compared with control group.

Tab 2. Effects of Bay K8644 and capsazepine on capsaicin-induced changes in transmembrane action potential in human atrial fibers

Group	MDP/mV	APA/mV	V_{max}/V·s^{-1}	VDD/mV·s^{-1}	RPF/min^{-1}	APD_{90}/ms
Control	(－58.7±9.6)	(64.2±7.0)	(12.6±3.8)	(16.0±5.7)	(52.4±10.0)	(254±31)
Capsaicin	－2.1±2.6	－3.8±2.3*	－2.3±0.8**	－5.1±2.4**	－13.9±10.0*	－20±9**
Bay K8644	－2.0±2.2	5.7±3.5*	8.7±7.0*	9.9±3.7**	14.5±5.9**	－3±6
Bay K8644 + capsaicin	－1.6±4.1	－0.5±3.7	－1.0±2.9	－2.3±3.8	－6.7±12.1	－11±14
Control	(-55.8±4.3)	(60.4±3.3)	(14.7±3.6)	(13.7±4.2)	(44.5±7.5)	(239±34)
Capsaicin	－1.2±4.5	－4.8±1.2**	－4.8±2.2**	－6.0±4.2*	－17.0±7.4**	－24±10**
Capsazepine	－0.2±1.3	0.5±1.4	－0.0±1.3	－0.5±0.6	－2.4±4.0	0±8
Capsazepine + capsaicin	0.7±1.3	－5.2±3.8**	－3.6±2.3*	－7.9±3.6**	－15.4±5.9**	－27±18**

Action potentials were recorded before (control) and 15 min after capsaicin 10 μmol·L^{-1} was applied; when washing off capsaicin, the atrial fibers were treated with Bay K8644 0.5 μmol·L^{-1} or capsazepine 10 μmol·L^{-1} for 10 min and AP were recorded; then capsaicin 10 μmol·L^{-1} were added again and AP were recorded 15 min later. $\bar{x} \pm s$, $n = 6$. * $P < 0.05$, ** $P < 0.01$, compared with control group by paired t test.

capsaicin could exert inhibitory actions on the automaticity of human atrial special fibers and a concentration-dependent decrease in APA, V_{max}, VDD and RPF. The change in RPF was accompanied by a decrease in the VDD, which indicated that the inhibitory effects of capsaicin were mainly attributed to the reduction in VDD. Thus we presumed that the above effects of capsaicin might be attributed to the reduction of I_{Ca}. Our presumption was substantiated by the finding that application of L-type Ca^{2+} channel agonist Bay K8644 antagonized the inhibitory effects of capsaicin. We also found that APD was shortened by capsaicin. Li, *et al*[13] reported that I_{Ca} contributed importantly to APD of human atrial special fibers. The above action on APD might result from the reduction in I_{Ca} and deserved further investigation.

It has been reported that capsaicin receptor (i.e. VR_1), a distant relative of the transient release potential family of stored-operated calcium channels, is expressed almost exclusively in primary sensory neurons, but there are also non-neuronal VR[14,15]. Recently evidence has been presented that VR_1 is expressed by non-neuronal cells of rat cardiomyocytes during development[16]. Human VR_1 is expressed in dorsal root ganglion as an approximately 4.2 kilobase RNA, and is also expressed in the central nervous system and in the kidney[17]. We think it is possible that capsaicin can act directly on the heart *via* a cardiac VR. Capsazepine, a competitive capsaicin antagonist, is able to block the VR and inhibits capsaicin-induced responses[18]. However, in our experiment, capsazepine failed to abolish the electrophysiological effects of capsaicin on human atrial fibers, suggesting that VR_1 not mediate the inhibitory effects of capsaicin.

In conclusion, this study for the first time established that capsaicin exerted a negative chronotropic action and accelerated the repolarization of human atrial specialized fibers. These effects are likely due to reduction in calcium influx and not mediated by VR_1.

4 REFERENCES:

[1] Szolcsanyi J, Oroszi G, Nemeth J, Szilvassy Z, Blasig IE, Tosaki A. Functional and biochemical evidence for capsaicin-induced neural endothelin release in isolated working rat heart[J]. *Eur J Pharmacol*, 2001, **419**(2－3):215－221.

[2] Zhou FW, Li YJ, Deng HW. Early and delayed protection by capsaicin against reperfusion injury in rat hearts[J]. *Acta Pharmacol Sin*(中国药理学报), 1999, **20**(10): 912－916.

[3] Castle NA. Differential inhibition of potassium currents in rat ventricular myocytes by capsaicin[J]. *Cardiovasc Res*, 1992, **26**(11):1137 – 1144.
[4] Milesi V, Rebolledo A, Alvis AG, Raingo J, Grassi de Gende AO. Voltage-activated sodium current is inhibited by capsaicin in rat atrial myocytes[J]. *Biochem Biophys Res Commun*, 2001, **282**(4):965 – 970.
[5] Li Q, Cheng YP, He RR. Electrophysiological effects of capsaicin on guinea pig papillary muscles[J]. *Acta Physiol Sin*(生理学报), 2003, **55**(5):511 – 515.
[6] Cheng YP, Wang YH, Cheng LP, He RR. Electrophysiologic effects of capsaicin on pacemaker cells in sinoatrial nodes of rabbits[J]. *Acta Pharmacol Sin*(中国药理学报), 2003, **24**(8):826 – 830.
[7] Li Q, Wu YM, He RR. Electrophysiological effects of capsaicin on spontaneous activity of rabbit atrioventricular node cells[J]. *Acta Physiol Sin*(生理学报), 2004, **56**(2):248 – 252.
[8] Gelband H, Bush HL, Rosen MR, Myerburg RJ, Hoffman BF. Electrophysiologic properties of isolated preparations of human atrial myocardium[J]. *Circ Res*, 1972, **30**(3):293 – 300.
[9] Mary-Rabine L, Hordof AJ, Danilo P Jr, Malm JR, Rosen MR. Mechanisms for impulse initiation in isolated human atrial fibers[J]. *Circ Res*, 1980, **47**(2):267 – 277.
[10] Fan ZZ, An RH, He RR. System of sampling and processing cardiac transmembrane potential by microcomputer[J]. *Chin J Phys Med*(中华物理医学杂志), 1991, **13**(1):39 – 42.
[11] Kodama I, Honjo H, Boyett MR. Are we lost in the labyrinth of the sinoatrial node pacemaker mechanism[J]? *J Cardiovasc Electrophysiol*, 2002, **13**(12):1303 – 1305.
[12] Mangoni ME, Couette B, Bourinet E, Platzer J, Reimer D, Striessnig J, *et al*. Functional role of L-type Cav1.3 Ca^{2+} channels in cardiac pacemaker activity[J]. *Proc Natl Acad Sci USA*, 2003, **100**(9):5543 – 5548.
[13] Li GR, Nattel S. Properties of human atrial I_{Ca} at physiological temperatures and relevance to action potential[J]. *Am J Physiol*, 1997, **272**(1 Pt 2):H227 – H235.
[14] Biro T, Brodie C, Modarres S, Lewin NE, Acs P, Blumberg PM. Specific vanilloid responses in C_6 rat glioma cells[J]. *Brain Res Mol Brain Res*, 1998, **56**(1 – 2):89 – 98.
[15] Biro T, Maurer M, Modarres S, Lewin NE, Brodie C, Acs G, *et al*. Characterization of functional vanilloid receptors expressed by mast cells[J]. *Blood*, 1998, **91**(4):1332 – 1340.
[16] Dvorakova M, Kummer W. Transient expression of vanilloid receptor subtype 1 in rat cardiomyocytes during development[J]. *Histochem Cell Biol*, 2001, **116**(3):223 – 225.
[17] Cortright DN, Crandall M, Sanchez JF, Zou T, Krause JE, White G. The tissue distribution and functional characterization of human VR1[J]. *Biochem Biophys Res Commun*, 2001, **281**(5):1183 – 1189.
[18] Bevan S, Hothi S, Hughes G, James IF, Rang HP, Shah K, *et al*. Capsazepine: a competitive antagonist of the sensory neuron excitant capsaicin[J]. *Br J Pharmacol*, 1992, **107**(2):544 – 552.

辣椒素对人心房肌的电生理效应

李　茜[1], 刘　苏[2], 陈子英[2], 刘宜先[1], 张文立[2], 赵　宏[2], 刘林立[2], 何瑞荣[1]
(1. 河北医科大学基础医学研究所生理室, 河北 石家庄　050017; 2. 河北医科大学附属第二医院心脏外科, 河北 石家庄　050000)

摘要: 目的　研究辣椒素对人心房肌的电生理效应及其作用机制。**方法**　应用经典玻璃微电极方法记录人心房肌特殊细胞的动作电位。**结果**　辣椒素(1 ~ 30 $\mu mol \cdot L^{-1}$)浓度依赖性地抑制人心房肌纤维的动作电位幅值,0 期最大除极速率,舒张期(4 相)除极化速率和起搏细胞放电频率,此外还缩短 90% 动作电位时程。应用 L-型钙通道开放剂 Bay K8644 (0.5 $\mu mol \cdot L^{-1}$)可拮抗辣椒素对人心房肌纤维的上述电生理效应,但辣椒素受体竞争性抑制剂 capsazepine(10 $\mu mol \cdot L^{-1}$)对辣椒素的效应并无影响。**结论**　辣椒素对人心房肌具有负性变时作用,并可缩短复极化时程。这些效应可能与其抑制钙离子内流有关。

关键词: 辣椒素; 心房; 电生理学; 钙

(本文编辑　董立春)