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Electrophysiologic effects of capsaicin on pacemaker cells in sinoatrial nodes of rabbits

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KEY WORDS capsaicin; sinoatrial node; Bay-K-8644; ruthenium red; isoproterenol; eletrophysiology

ABSTRACT

AIM: To study the electrophysiologic effects of capsaicin on isolated pacemaker cells in sinoatrial (SA) nodes of rabbits and its possible action mechanism(s). **METHODS:** Parameters of action potential (AP) in SA node were recorded using intracellular microelectrode technique. **RESULTS:** By perfusion with capsaicin (10 μmol/L), the amplitude of action potential (APA) and maximal rate of depolarization (V_{max}) were decreased from (55±4) mV to (49±4) mV ($P<0.05$) and from (2.4±0.5) V/s to (1.7±0.2) V/s ($P<0.05$). The velocity of diastolic (phase 4) depolarization (VDD) and rate of pacemaker firing (RPF) were decreased from (91±34) mV/s to (70±30) mV/s ($P<0.01$) and from (186±14) beat/min to (162±10) beat/min ($P<0.01$). The absolute value of maximal diastolic potential (MDP) was decreased from (49±3) mV to (44±2) mV ($P<0.01$). However, the duration of 90 % repolarization of action potential (APD_{90}) was prolonged from (149±21) ms to (167±27) ms ($P<0.01$). Pretreatment with ruthenium red (RR, 10 μmol/L), a vanilloid receptor (VR1) blocker, did not affect the effects of capsaicin on SA node cells. Both elevation of calcium concentration (5 mmol/L) in superfusate and application of L-type Ca^{2+} channel agonist Bay-K-8644 (0.5 μmol/L) antagonized the effects of capsaicin on pacemaker cells. β-adrenergic agonist isoproterenol (Iso, 20 nmol/L) inhibited the capsaicin-induced prolongation of repolarization and decrease of MDP. **CONCLUSION:** Capsaicin exerted a negative chronotropic action and induced a delayed repolarization of pacemaker cells in SA nodes of rabbits. These effects were likely due to reduction in calcium influx and/or potassium efflux, but were not mediated by VR1.

INTRODUCTION

Capsaicin, the main pungent ingredient in ‘ hot’ chili peppers, has long been known to influence cardiac functions. Capsaicin caused a long-lasting, positive inotropic and chronotropic effect on the spontaneously beating right atrium and a slight initial inhibition of ventricular contractility, which was followed by a marked stimulatory action[1]. Capsaicin produced a marked increase in coronary flow, a large positive chronotropic effect and a significant reduction in contractile strength[2]. These actions on heart were mediated by its interaction with a specific vanilloid receptor (VR1) on sensory nerve endings in cardiac muscles and opening of a non-selective cation channel, inducing the liberation of neuropeptides, most notably calcitonin gene-related peptide (CGRP), from the vanilloid-sensitive innervation of the heart[3-5]. Little is known, however, about the effects of capsaicin on pacemaker cells of isolated si-

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noatrial (SA) node. In the present study, we observed the electrophysiologic effects of capsaicin on SA node cells of rabbits and investigated the mechanism(s) involved.

MATERIALS AND METHODS

Preparation Rabbits of either sex (n=30, weighing 2.2 kg±0.2 kg, Grade II, Certificate No 04037, provided by Experimental Animal Center of Hebei Province) were killed with a single blow on the head and the hearts were quickly excised. The region of the right atrium bounded by the crista terminalis and the superior and inferior vena cava, and the interatrial septum were dissected free from the adjacent tissue[6] in Krebs-Henseleit (K-H) solution (0-4 °C). The preparations were pinned down on a thin silicon disc on the base of a perfusion chamber and equilibrated for 1 h. The K-H solution was prepared with deionized, distilled water and had the following composition (mmol/L): NaCl 118.0, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4$ 1.6, $CaCl_2$ 2.5, KH_2PO_4 1.2, and glucose 11.1. It was oxygenated with 95 % O_2 and 5 % CO_2 and maintained at (36.0±0.5) °C with pH of 7.40±0.03.

Electrical recording The transmembrane potentials were recorded by KCl (3 mol/L)-filled micropipettes (tip diameter less than 0.5 μm), coupled to a high input impedance amplifier (MEZ 8201, Nihon Kohden). The amplified signals were fed to the A/D converter and processed by a microcomputer. Maximal diastolic potential (MDP), amplitude of action potential (APA), 90 % of duration of action potential (APD_{90}), maximal rate of depolarization (V_{max}), rate of pacemaker firing (RPF), and velocity of diastolic (phase 4) depolarization (VDD) were analyzed with the system of sampling and processing cardiac transmembrane potential designed by our department[7].

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Drugs Drugs used in this study included capsaicin, ruthenium red, and Bay- K- 8644 (Sigma Chemical Co, USA) and isoproterenol (Tianfeng Pharmacy Co, China). Capsaicin was dissolved in distilled water containing 10 % ethanol and 1 % Tween-80, and then diluted to final concentration with saline. Bay-K-8644 was prepared as stock solution in alcohol and the final concentration of alcohol was 0.1 %. Ruthenium red was dissolved in distilled water.

Statistical analysis All data were presented as mean±SD. Statistical comparisons were performed using t test. Statistical significance was set at $P<0.05$.

RESULTS

Effect of capsaicin on transmembrane action potentials Compared with control groups, capsaicin (1-30 μmol/L) decreased VDD, RPF, and V_{max}, and prolonged APD_{90} in a concentration-dependent manner. Capsaicin 10 and 30 μmol/L also induced a significant reduction in APA and MDP (Tab 1, Fig 1). The changes in RPF induced by capsaicin paralleled to those of VDD. The above effects occurred after 10 min of superfusion of capsaicin and reached the peak within 20-30 min. The vehicle of capsaicin had no effect on parameters

Tab 1. Effects of capsaicin on transmembrane action potentials of rabbit sinoatrial node cells. *n*=6. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* control.

	MDP/mV	APA/mV	V_{max}/V· s^{-1}	VDD/mV· s^{-1}	APD_{90}/ms	RPF/beat· min^{-1}
Control	-49±3	55±4	2.4±0.5	91±34	149±21	186±14
Capsaicin (μmol/L)						
1	-48±4[a]	53±5[a]	2.1±0.3[a]	84±34[b]	154±19[b]	178±12[a]
3	-47±3[a]	51±4[a]	1.9±0.3[b]	77±35[b]	159±23[c]	171±11[b]
10	-44±2[c]	49±4[b]	1.7±0.2[b]	70±30[c]	167±27[c]	162±10[c]
30	-43±2[c]	47±4[c]	1.5±0.4[b]	59±29[c]	182±42[b]	151±17[c]

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.

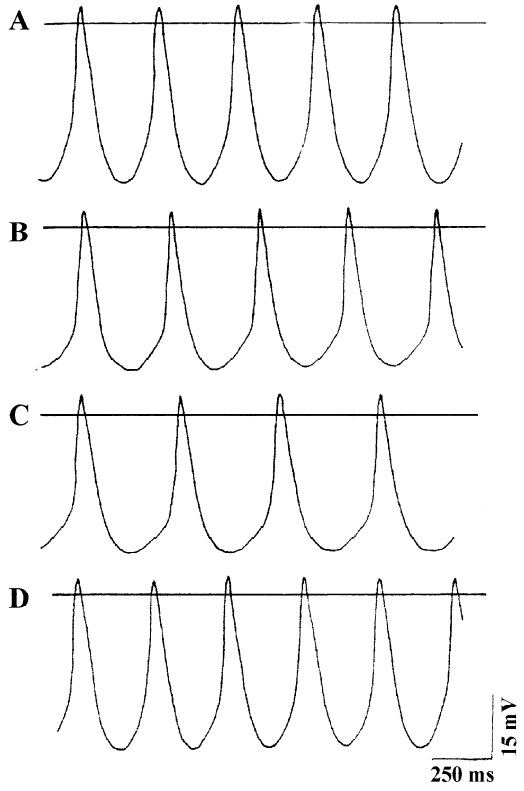

Fig 1. Effects of capsaicin on transmembrane action potentials of rabbit sinoatrial node cells. A: Control; B: Capsaicin 3 μmol/L; C: Capsaicin 30 μmol/L; D: Wash out.

of AP of pacemaker cells.

Effects of ruthenium red on the response of SA node cells to capsaicin Vanilloid receptor (VR1) blocker RR (10 μmol/L) had no effect on AP. Pretreatment with RR 10 μmol/L failed to affect the above mentioned effects induced by capsaicin 10 μmol/L (Tab 2).

Effects of Bay-K-8644 on capsaicin-induced changes of AP L-type calcium channel agonist Bay-K-8644 (0.5 μmol/L) significantly increased V_{max}, RPF, and VDD. Upon the application of Bay-K-8644, the effects of capsaicin (10 μmol/L) in V_{max}, RPF, and VDD were inhibited (Tab 2). The vehicle of Bay-K-8644 (0.1 % alcohol in superfusate) had no effect on parameters of AP of pacemaker cells.

Effects of high Ca^{2+} on the actions of capsaicin Elevation of Ca^{2+} concentration (5 mmol/L) in superfusate increased VDD, V_{max}, and APA, and blocked the inhibitory action of capsaicin 10 μmol/L (Tab 2).

Effects of isoproterenol on capsaicin-induced changes in MDP and repolarization of SA node cells β-adrenergic agonist Iso (20 nmol/L) not only increased V_{max}, RPF, and APA, but also significantly shortened APD_{90} and increased MDP. After pretreatment with Iso 20 nmol/L, the APD_{90}-prolonging and MDP-decreasing effects of capsaicin (10 μmol/L) were antagonized (Tab 2).

DISCUSSION

The present study showed that capsaicin concentration-dependently decreased V_{max}, VDD, RPF, APA and MDP, and prolonged APD_{90} of pacemaker cells in SA nodes of rabbits. It has been generally accepted that many of the capsaicin effects are mediated by vanilloid receptor (VR1). VR1, a distant relative of the transient release potential (TRP) family of stored-operated calcium channels, is expressed almost exclusively by primary sensory neurons involved in nociceptions and inflammation[8]. On the other hand, some capsaicin-induced effects did not follow typical features of vanilloid receptors[9]. The presence of a VR1-like expressed sequence tags (EST) clone in heart is in accor-

Tab 2. Effects of ruthenium red (RR, 10 μmol/L), Bay-K-8644 (Bay, 0.5 μmol/L), high Ca^{2+} (5 mmol/L), and isoproterenol (Iso, 20 nmol/L) on capsaicin (10 μmol/L)-induced changes on transmembrane action potentials of rabbit sinoatrial node cells. *n*=24. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* control.

	MDP/mV	APA/mV	V_{max}/V· s^{-1}	VDD/mV· s^{-1}	APD_{90}/ms	RPF/beat· min^{-1}
Control	-48±7	60±8	3.0±0.7	81±17	124±14	196±28
Capsaicin	-44±7[c]	53±8[c]	1.9±0.5[c]	59±22[c]	143±16[c]	166±21[c]
RR	-49±7[a]	60±7[a]	2.8±0.5[b]	77±12[a]	127±11[a]	189±23[a]
RR+capsaicin	-45±5[a]	54±7[b]	1.9±0.4[b]	59±19[c]	145±19[c]	164±18[c]
Control	-44±3	50±4	1.8±0.4	103±20	136±7	216±13
Capsaicin	-41±3[c]	45±4[b]	1.58±0.24[b]	75±4[b]	146±6[c]	193±9[c]
Bay	-44±3[a]	55±3[a]	2.5±0.4[c]	145±31[b]	134±5[a]	236±14[c]
Bay+capsaicin	-43±2[b]	49±3[a]	1.9±0.3[a]	107±15[a]	145±3[a]	212±13[a]
Control	-47±5	50±5	2.3±0.6	67±10	132±10	229±27
Capsaicin	-43±4[b]	47±5[a]	1.9±0.3[b]	49±10[c]	146±12[b]	188±30[b]
High Ca^{2+}	-48±5[a]	52±5[b]	3.4±1.0[b]	79±8[b]	129±12[a]	235±28[a]
High Ca^{2+}+capsaicin	-47±5[a]	51±4[a]	2.5±0.7[a]	71±9[a]	129±10[a]	230±24[a]
Control	-49±9	58±13	3.9±1.4	57±7	116±9	221±27
Capsaicin	-46±7[b]	55±14[b]	2.9±0.9[b]	50±4[a]	140±7[c]	169±34[b]
Iso	-54±9[c]	64±12[b]	6.3±2.5[b]	75±19[b]	104±6[c]	255±32[c]
Iso+capsaicin	-48±8[a]	58±13[a]	4.5±1.7[a]	63±14[a]	114±8[a]	238±28[a]

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

dance with the recognition of nonneuronal VRs[10,11]. The negative inotropic actions on cardiac muscles of high-dose (in μmol/L) of capsaicin was not due to the toxic effect of capsaicin[12]. In our experiment, RR, a vanilloid receptor blocker, failed to abolish the electrophysiologic effects of capsaicin on SA node, suggesting that VR1 might not mediate the inhibitory effects of capsaicin. High-dose of capsaicin might exert its action through the nonspecific mechanism.

It has been widely accepted that calcium currents play important roles in action potential upstroke and pacemaker depolarization of SA node cells[13,14]. Therefore, the inhibitory effects of capsaicin on V_{max}, VDD, RPF, and APA might be attributed to the reduction of I_{Ca}. Our presumption was substantiated by the following findings that elevation of calcium concentration in superfusate or application of L-type Ca^{2+} channel agonist Bay-K-8644 blocked the inhibitory effects of capsaicin.

In this study, maximal diastolic potential was decreased and action potential duration of pacemaker cells was prolonged as the concentration of capsaicin increased. It has been well known that I_K is the main ionic current which participates in repolarization of SA node[13], so the above action might be related to a reduction of potassium current. Castle reported that capsaicin increased the action potential duration of rat ventricular myocytes. The effect was associated with an inhibition of three distinct K^+ currents, I_{to}, I_K, and I_{Ki}[15]. In our study, β-adrenergic agonist isoproterenol increased V_{max}, VDD, and RPF of SA node cells. At the same time, Iso significantly shortened APD_{90} and increased MDP. The latter effects were due to the activation of I_K[16,17]. Iso inhibited the capsaicin-induced decrease of MDP and prolongation of repolarization of SA node cells, suggesting that capsaicin might possess the ability to inhibit I_K in SA node.

In summary, capsaicin exhibited inhibitory effects on pacemaker cells in SA nodes of rabbits, which may be attributed to reduction in calcium influx and/or potassium efflux and may be not mediated by VR1.

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Control	-49±3	55±4	2.4±0.5	91±34	149±21	186±14
Capsaicin (μmol/L)						
1	-48±4[a]	53±5[a]	2.1±0.3[a]	84±34[b]	154±19[b]	178±12[a]
3	-47±3[a]	51±4[a]	1.9±0.3[b]	77±35[b]	159±23[c]	171±11[b]
10	-44±2[c]	49±4[b]	1.7±0.2[b]	70±30[c]	167±27[c]	162±10[c]
30	-43±2[c]	47±4[c]	1.5±0.4[b]	59±29[c]	182±42[b]	151±17[c]

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.

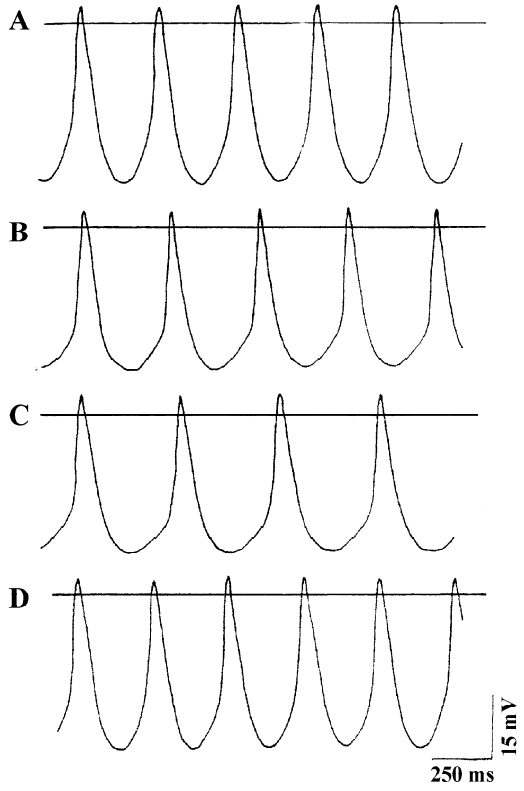

Fig 1. Effects of capsaicin on transmembrane action potentials of rabbit sinoatrial node cells. A: Control; B: Capsaicin 3 μmol/L; C: Capsaicin 30 μmol/L; D: Wash out.

of AP of pacemaker cells.

Effects of ruthenium red on the response of SA node cells to capsaicin Vanilloid receptor (VR1) blocker RR (10 μmol/L) had no effect on AP. Pretreatment with RR 10 μmol/L failed to affect the above mentioned effects induced by capsaicin 10 μmol/L (Tab 2).

Effects of Bay-K-8644 on capsaicin-induced changes of AP L-type calcium channel agonist Bay-K-8644 (0.5 μmol/L) significantly increased V_{max}, RPF, and VDD. Upon the application of Bay-K-8644, the effects of capsaicin (10 μmol/L) in V_{max}, RPF, and VDD were inhibited (Tab 2). The vehicle of Bay-K-8644 (0.1 % alcohol in superfusate) had no effect on parameters of AP of pacemaker cells.

Effects of high Ca^{2+} on the actions of capsaicin Elevation of Ca^{2+} concentration (5 mmol/L) in superfusate increased VDD, V_{max}, and APA, and blocked the inhibitory action of capsaicin 10 μmol/L (Tab 2).

Effects of isoproterenol on capsaicin-induced changes in MDP and repolarization of SA node cells β-adrenergic agonist Iso (20 nmol/L) not only increased V_{max}, RPF, and APA, but also significantly shortened APD_{90} and increased MDP. After pretreatment with Iso 20 nmol/L, the APD_{90}-prolonging and MDP-decreasing effects of capsaicin (10 μmol/L) were antagonized (Tab 2).

DISCUSSION

The present study showed that capsaicin concentration-dependently decreased V_{max}, VDD, RPF, APA and MDP, and prolonged APD_{90} of pacemaker cells in SA nodes of rabbits. It has been generally accepted that many of the capsaicin effects are mediated by vanilloid receptor (VR1). VR1, a distant relative of the transient release potential (TRP) family of stored-operated calcium channels, is expressed almost exclusively by primary sensory neurons involved in nociceptions and inflammation[8]. On the other hand, some capsaicin-induced effects did not follow typical features of vanilloid receptors[9]. The presence of a VR1-like expressed sequence tags (EST) clone in heart is in accor-

Tab 2. Effects of ruthenium red (RR, 10 μmol/L), Bay-K-8644 (Bay, 0.5 μmol/L), high Ca^{2+} (5 mmol/L), and isoproterenol (Iso, 20 nmol/L) on capsaicin (10 μmol/L)-induced changes on transmembrane action potentials of rabbit sinoatrial node cells. *n*=24. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* control.

	MDP/mV	APA/mV	V_{max}/V· s^{-1}	VDD/mV· s^{-1}	APD_{90}/ms	RPF/beat· min^{-1}
Control	-48±7	60±8	3.0±0.7	81±17	124±14	196±28
Capsaicin	-44±7[c]	53±8[c]	1.9±0.5[c]	59±22[c]	143±16[c]	166±21[c]
RR	-49±7[a]	60±7[a]	2.8±0.5[b]	77±12[a]	127±11[a]	189±23[a]
RR+capsaicin	-45±5[a]	54±7[b]	1.9±0.4[b]	59±19[c]	145±19[c]	164±18[c]
Control	-44±3	50±4	1.8±0.4	103±20	136±7	216±13
Capsaicin	-41±3[c]	45±4[b]	1.58±0.24[b]	75±4[b]	146±6[c]	193±9[c]
Bay	-44±3[a]	55±3[a]	2.5±0.4[c]	145±31[b]	134±5[a]	236±14[c]
Bay+capsaicin	-43±2[b]	49±3[a]	1.9±0.3[a]	107±15[a]	145±3[a]	212±13[a]
Control	-47±5	50±5	2.3±0.6	67±10	132±10	229±27
Capsaicin	-43±4[b]	47±5[a]	1.9±0.3[b]	49±10[c]	146±12[b]	188±30[b]
High Ca^{2+}	-48±5[a]	52±5[b]	3.4±1.0[b]	79±8[b]	129±12[a]	235±28[a]
High Ca^{2+}+capsaicin	-47±5[a]	51±4[a]	2.5±0.7[a]	71±9[a]	129±10[a]	230±24[a]
Control	-49±9	58±13	3.9±1.4	57±7	116±9	221±27
Capsaicin	-46±7[b]	55±14[b]	2.9±0.9[b]	50±4[a]	140±7[c]	169±34[b]
Iso	-54±9[c]	64±12[b]	6.3±2.5[b]	75±19[b]	104±6[c]	255±32[c]
Iso+capsaicin	-48±8[a]	58±13[a]	4.5±1.7[a]	63±14[a]	114±8[a]	238±28[a]

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

dance with the recognition of nonneuronal VRs[10,11]. The negative inotropic actions on cardiac muscles of high-dose (in μmol/L) of capsaicin was not due to the toxic effect of capsaicin[12]. In our experiment, RR, a vanilloid receptor blocker, failed to abolish the electrophysiologic effects of capsaicin on SA node, suggesting that VR1 might not mediate the inhibitory effects of capsaicin. High-dose of capsaicin might exert its action through the nonspecific mechanism.

It has been widely accepted that calcium currents play important roles in action potential upstroke and pacemaker depolarization of SA node cells[13,14]. Therefore, the inhibitory effects of capsaicin on V_{max}, VDD, RPF, and APA might be attributed to the reduction of I_{Ca}. Our presumption was substantiated by the following findings that elevation of calcium concentration in superfusate or application of L-type Ca^{2+} channel agonist Bay-K-8644 blocked the inhibitory effects of capsaicin.

In this study, maximal diastolic potential was decreased and action potential duration of pacemaker cells was prolonged as the concentration of capsaicin increased. It has been well known that I_K is the main ionic current which participates in repolarization of SA node[13], so the above action might be related to a reduction of potassium current. Castle reported that capsaicin increased the action potential duration of rat ventricular myocytes. The effect was associated with an inhibition of three distinct K^+ currents, I_{to}, I_K, and I_{Ki}[15]. In our study, β-adrenergic agonist isoproterenol increased V_{max}, VDD, and RPF of SA node cells. At the same time, Iso significantly shortened APD_{90} and increased MDP. The latter effects were due to the activation of I_K[16,17]. Iso inhibited the capsaicin-induced decrease of MDP and prolongation of repolarization of SA node cells, suggesting that capsaicin might possess the ability to inhibit I_K in SA node.

In summary, capsaicin exhibited inhibitory effects on pacemaker cells in SA nodes of rabbits, which may be attributed to reduction in calcium influx and/or potassium efflux and may be not mediated by VR1.

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