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Electrophysiological effects of phytoestrogen genistein on pacemaker cells in sinoatrial nodes of rabbits

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KEY WORDS genistein; sinoatrial node; electrophysiology

ABSTRACT

AIM: To study the electrophysiological effects of genistein (GST) on pacemaker cells in sinoatrial (SA) nodes of rabbits. **METHODS**: Parameters of action potential (AP) in SA node were recorded using intracellular microelectrode technique. **RESULTS**: GST (10 - 150 μmol/L) not only decreased the amplitude of action potential (APA), maximal rate of depolarization (V_{max}) [from (6.2 ± 2.8) to (2.8 ± 1.4) V/s, $P < 0.01$], velocity of diastolic (phase 4) depolarization (VDD) [from (55 ± 14) to (38 ± 8) mV/s, $P < 0.01$], and rate of pacemaker firing (RPF) [from (154 ± 23) to (107 ± 25) beat/min, $P < 0.01$], but also prolonged duration of 90 % repolarization of action potential (APD_{90}) in a concentration-dependent manner. Both elevation of calcium concentration (5 mmol/L) in superfusate and application of L-type Ca^{2+} channel agonist Bay K8644 (0.5 μmol/L) reversed the inhibitory effects of GST on pacemaker cells, while pretreatment with N^G-nitro-*L*-arginine methyl ester (*L*-NAME, 1 mmol/L), an NO synthase inhibitor, failed to block the electrophysiological effects of GST. **CONCLUSION**: GST 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 potassium efflux, but had no association with NO release.

INTRODUCTION

Phytoestrogens are plant-derived diphenolic compounds which are structurally and functionally similar to estradiol. Accumulating evidence indicates that phytoestrogens may confer cardiovascular protection[1-3]. Genistein (GST), one of the most well-known phytoestrogens, is an isoflavone which is also proved to be a specific inhibitor of protein tyrosine kinase (PTK)[4]. Recently, we found that GST shortened duration of action potential in papillary muscles of normal guinea pig and also decreased maximal velocity of phase 0 depolarization in partially depolarized papillary muscles. These effects were likely due to a decrease of calcium inflow[5]. Little is known, however, about the effects of GST on pacemaker cells of sinoatrial (SA) node. In the present study, we observed the electrophysiological effects of GST on SA nodes of rabbits and investigated the mechanism(s) involved.

MATERIALS AND METHODS

Preparation Male rabbits ($n = 25$, weighing 2.6 kg ± 0.3 kg, Grade Ⅱ, Certificate № 04037, provided by Experimental Animal Center of Hebei Province) were stunned by heavy blow on the heads 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 was dissected free from the adjacent tissue in Krebs-Henseleit (K-H) solution (0 - 4 ℃). 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) with K-H solution (36.0 ± 0.5) ℃ of the following composition: 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 mmol/L. The K-H solution was saturated by a mixture of 95 % O_2 and 5 % CO_2 and the pH was 7.39 ± 0.03.

Electrophysiological measurements Transmembrane action potentials were recorded from pacemaker cells of SA node with a glass microelectrode filled with KCl 3 mol/L (a tip resistance of 10 - 20 MΩ), coupled

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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 duration of 90 % repolarization of action potential (APD_{90}) were analyzed with the program designed by our department[6]. Parameters were stored in the microcomputer for later analysis.

Experimental protocols Action potentials (AP) were recorded after an equilibration time of 1 h. The experiments consisted of 4 groups: (1) Electrophysiological effects of GST on SA node pacemaker cells. After recording of 3 control AP, GST 10, 50, 100, 150 μmol/L were applied, respectively. AP were then recorded at 1, 5, 10, 20, 30 min after application of GST; (2) Effects of Bay K8644 (0.5 μmol/L) on GST (100 μmol/L)-induced changes in AP of pacemaker cells. The effects of GST alone were observed firstly. Then after superfusion of Bay K8644 (0.5 μmol/L) for 15 min, GST 100 μmol/L was added to the superfusate containing Bay K8644 and AP were recorded; (3) Effects of high Ca^{2+} (5 mmol/L) on the response of pacemaker cells to GST (100 μmol/L). The effects of GST alone were observed firstly. Then normal K-H solution was replaced by high Ca^{2+} (5 mmol/L) K-H solution for 20 min. Afterwards, GST 100 μmol/L was administrated and AP were recorded; (4) Effects of *L*-NAME (1 mmol/L) on the action of GST (100 μmol/L). The effects of GST alone were observed firstly. Then after pretreatment with *L*-NAME 1 mmol/L, GST 100 μmol/L was applied and AP were recorded. In each experiment, the preparation was washed with K-H solution after application of drugs to observe the recovery of AP.

Drugs Drugs used in this study included GST, Bay K8644, *L*-NAME (Sigma Chemical Co, USA). GST and Bay K8644 were prepared as stock solutions in Me_2SO and alcohol, respectively. Final concentration of Me_2SO and alcohol was 0.05 % and 0.1 %, respectively. *L*-NAME was dissolved in distilled water.

Statistical analysis All data were presented as $\bar{x} \pm s$. The analysis of data for significance was performed by Student's *t* test. Difference of $P < 0.05$ was considered significant.

RESULTS

Effect of GST on transmembrane action potential Compared with control groups, GST (10 – 150 μmol/L) decreased VDD, RPF, and V_{max} in a concentration-dependent manner. GST 100 and 150 μmol/L prolonged APD_{90}, while GST 150 μmol/L also induced a significant reduction in APA (Tab 1, Fig 1). The changes in RPF induced by GST paralleled to those of VDD. The above effects occurred after 5 min of superfusion of GST and reached the peak within 15 – 20 min. The vehicle of GST (0.05 % Me_2SO in superfusate) showed no effect on parameters of AP of pacemaker cells.

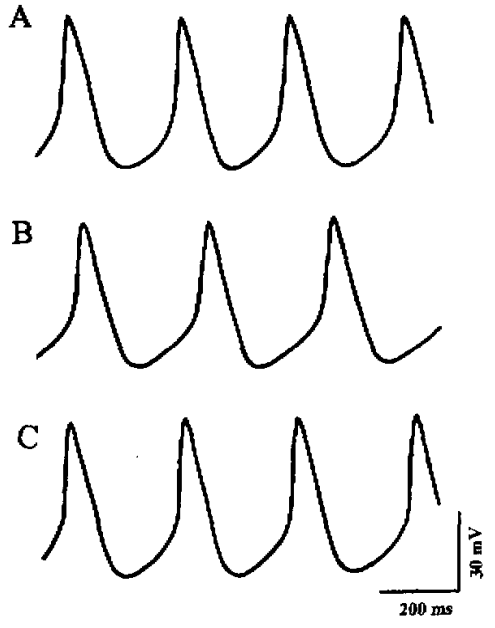

Fig 1. Original recording showing the effects of GST on transmembrane action potentials of rabbit sinoatrial node cells. A: Control; B: GST 100 μmol/L; C: Wash out.

Effects of Bay K8644 on GST-induced changes on AP L-type calcium channel agonist Bay K8644 (0.5 μmol/L) significant increased VDD, RPF, and V_{max}. Upon the application of Bay K8644, the effects of GST (100 μmol/L) were reversed (Tab 2). The vehicle of Bay K8644 (0.1 % alcohol in superfusate) had no effect on parameters of AP of pacemaker cells.

Tab 1. Effects of genistein (GST) on transmembrane action potentials of rabbit sinoatrial node cells. $n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

	MDP/mV	APA/mV	V_{max}/V·s^{-1}	VDD/mV·s^{-1}	RPF/beat·min^{-1}	APD_{90}/ms
Control	−65 ± 6	75 ± 7	6.2 ± 2.8	55 ± 14	154 ± 23	145 ± 11
GST/μmol·L^{-1}						
10	−65 ± 6^a	73 ± 9^a	5.9 ± 3.0^a	52 ± 15^a	150 ± 25^a	145 ± 12^a
50	−64 ± 5^a	70 ± 8^a	4.5 ± 1.9^b	45 ± 14^b	147 ± 24^c	152 ± 13^a
100	−64 ± 5^a	69 ± 8^a	3.4 ± 1.6^b	42 ± 10^c	128 ± 23^c	158 ± 16^b
150	−62 ± 7^a	65 ± 8^c	2.8 ± 1.4^c	38 ± 8^c	107 ± 25^c	167 ± 17^c

Tab 2. Effects of Bay K8644 (Bay, 0.5 μmol/L), high Ca^{2+} (5 mmol/L), and *L*-NAME (1 mmol/L) on genistein (GST, 100 μmol/L)-induced changes on transmembrane action potentials of rabbit sinoatrial node cells. $n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

	MDP/mV	APA/mV	V_{max}/V·s^{-1}	VDD/mV·s^{-1}	RPF/beat·min^{-1}	APD_{90}/ms
Control	−58 ± 3	68 ± 5	4.1 ± 2.3	65 ± 16	143 ± 21	157 ± 14
GST	−56 ± 5^a	66 ± 6^a	2.9 ± 2.6^b	51 ± 18^b	122 ± 20^c	169 ± 15^b
Bay	−56 ± 4^a	68 ± 4^a	5.8 ± 3.0^b	76 ± 18^c	163 ± 20^b	156 ± 16^a
Bay + GST	−56 ± 5^a	64 ± 6^a	3.7 ± 2.5^a	62 ± 19^a	140 ± 19^a	163 ± 24^a
Control	−60 ± 6	72 ± 7	6.3 ± 2.8	52 ± 3	131 ± 13	151 ± 12
GST	−58 ± 7^a	68 ± 8^a	4.1 ± 2.2^b	40 ± 5^c	112 ± 14^c	159 ± 10^a
High Ca^{2+}	−60 ± 6^a	74 ± 9^a	8.9 ± 3.8^b	64 ± 8^b	140 ± 15^b	151 ± 14^a
High Ca^{2+} + GST	−60 ± 7^a	69 ± 9^a	6.1 ± 2.5^a	46 ± 3^b	125 ± 14^b	152 ± 10^a
Control	−62 ± 5	74 ± 5	4.8 ± 2.6	45 ± 9	132 ± 8	162 ± 11
GST	−61 ± 4^a	68 ± 8^b	3.6 ± 2.2^b	32 ± 10^c	118 ± 8^c	171 ± 14^b
L-NAME	−60 ± 3^a	74 ± 6^a	4.7 ± 2.5^a	43 ± 9^a	131 ± 9^a	164 ± 10^a
L-NAME + GST	−59 ± 5^a	68 ± 8^b	3.5 ± 2.3^b	31 ± 9^c	116 ± 9^c	178 ± 20^b

Effects of high Ca^{2+} on the response of pacemaker cells to GST Elevation of Ca^{2+} concentration (5 mmol/L) in superfusate increased VDD, RPF, and V_{max}, and reversed the inhibitory action of GST 100 μmol/L (Tab 2).

Effects of *L*-NAME on the action of GST NO synthase inhibitor *L*-NAME (1 mmol/L) *per se* had no effect on AP. With the pretreatment of *L*-NAME 1 mmol/L, GST 100 μmol/L still decreased VDD, RPF, and V_{max} (Tab 2).

DISCUSSION

The present study showed that GST could exert inhibitory actions on the automaticity of pacemaker cells in SA nodes of rabbits, and decrease V_{max}, VDD, RPF, and APA in a concentration-dependent manner. It has been widely accepted that calcium currents play an important role in pacemaker depolarization[7,8]. Thus we presumed that the above effects of GST 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 K8644 reversed the inhibitory effects of GST.

Phytoestrogens are structurally similar to estrogens and may bind estrogen receptors to exhibit estrogen-like behavior[9]. Besides, evidence has been presented that NO release induced by estrogen could in part be responsible for its non-genomic actions in cardiovascular system[10]. On the other hand, Figtree *et al* found that inhibition of NO synthase had no effect on GST-induced relaxation of isolated rabbit coronary artery rings[3]. In our experiment, *L*-NAME, an inhibitor of NO synthase, failed to abolish the electrophysiological effects of GST on SA node, suggesting that NO release might not be

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involved.

In this study, action potential duration of pacemaker cells was prolonged as the concentration of GST increased. It has been well known that I_k is the main ionic current which participates in repolarization of SA node, so the above action might be related to a reduction of potassium current. Washizuka *et al* reported that GST suppressed delayed-rectifier K current in guinea pig ventricular myocytes[11]. Nevertheless, the involvement of other potassium currents can not be excluded and this issue merits further investigation by patch-clamp technique.

In conclusion, GST exerts a negative chronotropic action and induces a delayed repolarization of pacemaker cells in SA nodes of rabbits. These effects are likely due to reduction in calcium influx and potassium efflux, but have no association with NO release.

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植物性雌激素金雀异黄素对家兔窦房结起搏细胞的电生理效应

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关键词 金雀异黄素；窦房结；电生理学

目的：研究植物性雌激素金雀异黄素（genistein，GST）对家兔窦房结起搏细胞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。**结果**：GST(10-150 μmol/L)不仅以剂量依赖性方式抑制窦房结起搏细胞的零相最大上升速度（V_{max}），舒张期除极速度（VDD），起搏放电频率（RPF）和动作电位幅度（APA），而且延长复极化 90 % 时间（APD_{90}）。提高灌流液中钙离子浓度以及应用 L 型钙通道开放剂 Bay K8644 (0.5 μmol/L)均可逆转 GST 对起搏细胞的上述电生理效应，但 NO 合酶阻断剂 *L*-NAME (1 mmol/L)对 GST 的效应并无影响。**结论**：GST 对家兔窦房结具有负性变时作用，并可延长复极化时程。这些效应可能与其抑制钙离子内流及钾离子外流有关。

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