ISSN1671-4083
Shanghai Institute of Materia Medica
Chinese Academy of Sciences
http://www.ChinaPhar.com

Effects of phytoestrogen genistein on electrophysiology of human atrial fibers

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KEY WORDS genistein; atrium; electrophysiology; calcium

ABSTRACT

AIM: To study the electrophysiological effects of phytoestrogen genistein (GST) on human atrial fibers. **METHODS:** Parameters of action potential (AP) in human atrial special fibers were recorded using standard intracellular microelectrode technique. **RESULTS:** GST (10-100 μmol/L) decreased the velocity of diastolic (phase 4) depolarization (VDD) and rate of pacemaker firing (RPF), besides, GST (100 μmol/L) shortened the duration of 90 % repolarization of action potential (APD_{90}). L-type Ca^{2+} channel agonist Bay K8644 (0.5 μmol/L) antagonized the inhibitory effects of GST on human atrial fibers, while pretreatment of the fibers with N^G-nitro-*L*-arginine methyl ester (*L*-NAME, 1 mmol/L), an NO synthase inhibitor, failed to affect the electrophysiological effects of GST. **CONCLUSION:** GST exerted a negative chronotropic action and induced an accelerated repolarization of human atrial fibers. These effects were likely due to reduction in calcium influx.

INTRODUCTION

Phytoestrogens are plant-derived diphenolic compounds structurally and functionally similar to estradiol. An 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 have found that GST 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, GST also exerted a negative chronotropic action and induced a delayed repolarization of pacemaker cells in sinoatrial nodes of rabbits[6]. However, the effects of GST 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[7,8]. The present study was undertaken to investigate the electrophysiological effects of GST on human atrial specialized fibers and its action mechanism(s).

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Received 2001-11-08 Accepted 2002-05-30

MATERIALS AND METHODS

Preparation Small pieces (<1 cm^2) of atrial myocardium from anterior free wall of the right atrium were excised from the hearts of 19 patients undergoing corrective open heart surgery as part of the cannulation technique for cardiopulmonary bypass. Prior to surgery, informed consent was obtained. All patients were under 16 years old and suffered from congenital heart diseases including ventricular septal defects, 12; atrial septal defects, 3; Fallot's tetralogy, 2; Fallot's trilogy, 1; pulmonary stenosis, 1. 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 silicon rubber on the base of a perfusion chamber and equilibrated for 1 h. The preparation was superfused (4 mL/min) with Tyrode's solution (36.0±0.5℃) of the following composition: NaCl 137, $NaHCO_3$ 12, NaH_2PO_4 1.8, $MgCl_2$ 0.5, $CaCl_2$ 2.7, KCl 4, and dextrose 5.5 mmol/L. The Tyrode's 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 human atrial fibers with a glass microelectrode filled with 3 mol/L 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 duration of 90 % repolarization of action potential (APD_{90}) were analyzed with the program designed by our department[9]. Parameters were stored in the microcomputer for later analysis.

Experimental protocols AP was recorded after an equilibration time of 60 min. The experiments consisted of 3 groups: (1) Effect of GST on the electrophysiology of human atrial fibers. After recording of 3 control AP, GST 10, 50, and 100 μmol/L were applied, respectively. AP were recorded at 1, 5, 10, and 20 min after application of GST; (2) Effects of Bay K8644 (0.5 μmol/L) on GST (50 μmol/L)-induced changes in AP of pacemaker cells. After superfusion of Bay K8644 (0.5 μmol/L) for 15 min, GST (50 μmol/L) was added to the superfusate containing Bay K8644 and AP were recorded; (3) Effects of *L*-NAME (1 mmol/L) on the action of GST (50 μmol/L). After pretreatment of the fibers with *L*-NAME (1 mmol/L), GST (50 μmol/L) was applied and AP was recorded. In each experiment, the preparation was washed with Tyrode's solution after application of drugs to observe the recovery of AP.

Drugs Drugs used in this study included GST, Bay K8644, and *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 mean±SD. The analysis of data for significance was performed by Student's *t* test. Difference of $P<0.05$ was considered significant.

RESULTS

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.

Effects of GST on transmembrane action potential GST (10, 50, 100 μmol/L) decreased VDD and RPF. GST at 10 and 50 μmol/L induced marked decrease in V_{max}, but at 100 μmol/L it had no effect on V_{max}. Furthermore, GST 100 μmol/L also shortened APD_{90} (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 10-15 min. The vehicle of GST (0.05 % Me_2SO in superfusate) showed no effect on parameters of AP of pacemaker cells.

Effects of Bay K8644 on GST-induced changes on AP L-type calcium channel agonist Bay K8644 (0.5 μmol/L) significantly increased VDD and RPF. Pretreament of the fibers with Bay K8644 abolished the

Tab 1. Effects of genistein (GST) on transmembrane action potentials of human atrial fibers. *n*=5. 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}	RPF/min^{-1}	APD_{90}/ms
Control	-52±2	65±5	4.2±1.3	9.1±2.8	38±5	348±71
GST/μmol·L^{-1}						
10	-51.4±1.1[a]	62±6[a]	3.6±1.3[c]	6.4±1.6[b]	32±3[b]	337±53[a]
50	-52.8±2.3[a]	61±5[a]	3.6±1.3[b]	4.7±1.3[c]	28±6[c]	332±17[a]
100	-53±3[a]	66±4[a]	4.5±1.7[a]	4.4±1.3[c]	21.6±1.7[c]	296±9[b]

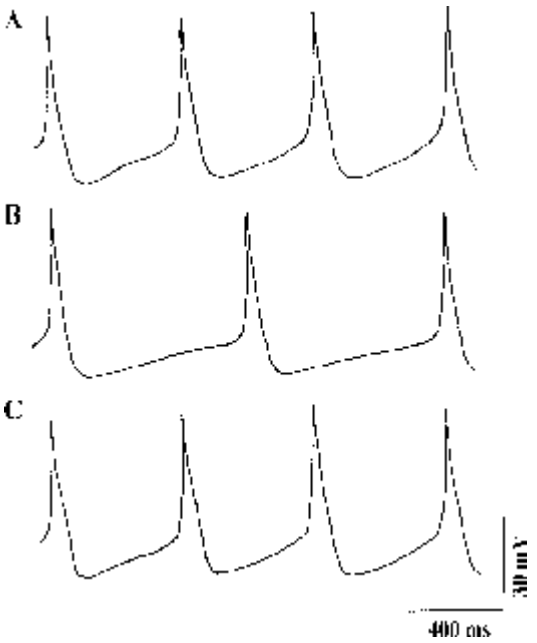

Fig 1. Original recording showing the effects of GST on transmembrane action potentials of human atrial fiber. A: Control; B: GST 50 μmol/L; C: Wash out.

effects of GST (50 μmol/L) (Tab 2). The vehicle of Bay K8644 (0.1 % alcohol in superfusate) had no effect on parameters of AP of pacemaker cells.

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 (50 μmol/L) still decreased VDD and RPF (Tab 2).

DISCUSSION

The present study showed that GST could exert inhibitory actions on the automaticity of human atrial special fibers, and decrease VDD and RPF in a concentration-dependent manner. The change in RPF was accompanied by a decrease in the VDD, which indicated that the inhibitory effects of GST were mainly attributed to the reduction in VDD. It has been widely accepted that calcium current plays an important role in pacemaker depolarization[8,10]. Thus we presumed that the above effects of GST might be attributed to the re-

Tab 2. Effects of Bay K8644 (Bay, 0.5 μmol/L) and *L*-NAME (1 mmol/L) on genistein (GST, 50 μmol/L)-induced changes of transmembrane action potentials in human atrial fibers. *n*=5. 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}	RPF/min^{-1}	APD_{90}/ms
Control	-53±3	63±8	2.5±0.8	6.6±2.2	34±3	322±50
GST	-55±3[a]	59±6[a]	2.1±0.6[b]	3.9±1.6[b]	24±6[c]	312±55[a]
Bay	-54.8±2.4[a]	69±7[b]	3.2±0.7[a]	8±3[b]	43±10[c]	311±55[a]
Bay+GST	-55±5[a]	66±10[a]	3.1±1.3[a]	5.2±0.8[a]	30±8[a]	315±71[a]
Control	-55.4±2.1	64±6	3.0±0.6	11±3	48±6	345±88
GST	-57±4[a]	61±8[a]	2.6±2.2[b]	6±4[c]	37±6[c]	329±85[a]
L-NAME	-56±3[a]	63±6[a]	2.7±0.5[a]	14±4[a]	47±6[a]	354±124[a]
L-NAME+GST	-53.6±2.3[a]	61±4[a]	2.8±1.2[a]	7±2[c]	33±8[c]	320±90[a]

duction 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 GST. We also found that APD was shortened as the concentration of GST was increased. Since Li and Nattel reported that I_{Ca} contributed importantly to APD of human atrial fibers[11], the above action on APD might result from the reduction in I_{Ca}. On the other hand, it was unexpected to find that the inhibitory effects of GST on V_{max} were reversed when the concentration was increased to 100 μmol/L. This effects might be the result of unspecific activation of some inward ion currents and deserved being further investigated by patch clamp technique.

Phytoestrogens are structurally similar to estrogens and may bind estrogen receptors to exhibit estrogen-like behavior[12]. Besides, evidence has been presented that NO release induced by estrogen could in part be responsible for its non-genomic actions in cardiovascular system[13]. Mishra *et al* showed that the relaxation of rat arteries by phytoestrogen genistein and daidzain was largely endothelium dependent and NO release was involved in the relaxation[14]. 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 human atrial fibers, suggesting that NO release might not be involved.

In conclusion, this study for the first time established that GST exerted a negative chronotropic action on human atrial special fibers. These effects are likely due to reduction in calcium influx.

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植物性雌激素金雀异黄素对人心房肌的电生理效应

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关键词 金雀异黄素；心房肌；电生理学；钙

目的：研究植物性雌激素金雀异黄素(genistein, GST)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．结果：GST (10-100 μmol/L)**抑制人心房肌纤维的舒张期(4 相)除极化速率(VDD)和起搏细胞放电频(RPF)，此外，**GST (100 μmol/L)**缩短APD_{90}．应用L型钙通道开放剂**Bay K8644 (0.5 μmol/L)**可拮抗GST对人心房肌纤维的上述电生理效应，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．**

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