Inhibition of Kv4.3 by genistein via a tyrosine phosphorylation-independent mechanism

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Genistein, an isoflavone compound isolated from the fermentation broth of Pseudomonas, has been characterized as a PTK inhibitor because it potently and selectively inhibits both receptor and nonreceptor tyrosine kinases. For example, genistein was reported to strongly inhibit the tyrosine kinase activity of the epidermal growth factor receptors, pp60v-src and PP110gag-fes, but have a much weaker effect on serine/threonine kinases, protein kinases A and C (3, 4). Since protein tyrosine phosphorylation plays an important role in modulating the activity of a variety of ion channels in excitable cells (19, 23, 33), genistein has been widely used as a pharmacological drug to monitor PTK involvement in the modulation of ion channels from different biological preparations. Using genistein, the PTK-dependent pathway was shown to be involved in the suppression of both the slow delayed-rectifier K+ current in guinea pig ventricular myocytes and the voltage-gated K+ channels expressed in Xenopus oocytes and human embryonic kidney cells (17, 26). Moreover, transient outward K+ currents were inhibited by genistein via PTK-dependent mechanisms in human atrial and rat ventricular myocytes (12, 28, 38). Additional studies show that genistein markedly reduced the amplitude of a slowly inactivating delayed rectifier current and, to a lesser extent, that of a transient K+ current in mouse Schwann cells (31). This action was accompanied by a decrease in tyrosine phosphorylation of the Kv1.4, Kv1.5, and Kv2.1 channel proteins (31). However, ever-increasing data show that genistein has other pharmacological activities, including direct action on ion channels through a PTK-independent mechanism. Via a PTK-independent pathway, genistein directly inhibited several K+ channels: voltage-gated K+ channels in the pulmonary arterial cells of rats and rabbits; a cardiac delayed-rectifier K+ current in the ventricular cells of the guinea pig; and a cloned human, A-type hKv1.4 in Chinese hamster ovary (CHO) cells (34, 39, 43). Genistein also has been shown to directly inhibit Ca2+ channels in vascular smooth muscle cells isolated from the artery of a rabbit ear (40).

Genistein has been widely used as a valuable pharmacological tool to study the PTK signaling pathway in electrophysiological studies. In the present study, we investigated the effects of genistein on cloned Kv4.3 channels expressed in CHO cells using a patch-clamp technique to determine the...
direct modulation of Kv4.3 by genistein via a PTK-independent manner.

MATERIALS AND METHODS

Stable transfection and cell culture. The Kv4.3 cDNA was stably transfected into CHO cells (American Type Culture Collection, Manassas, VA) using the lipofectamine reagent (Invitrogen, Grand Island, NY), as described previously (2, 30). CHO cells were cultured in Iscove’s modified Dulbecco’s medium (Invitrogen), supplemented with 10% fetal bovine serum, 2 mM glutamine, 0.1 mM hypoxanthine, and 0.01 mM thymidine, under a 95% humidified air-5% CO2 environment at 37°C. Transfected cells were exposed to 500 μg/ml gentamicin (Invitrogen), and antibiotic-resistant cells were selected and maintained in fresh Iscove’s modified Dulbecco’s medium containing gentamicin. By using a brief trypsin/EDTA (Invitrogen) treatment, transfected CHO cells were passed every 4–5 days and were seeded onto glass coverslips (diameter: 12 mm, Fisher Scientific, Pittsburgh, PA) in a petri dish 24 h before use. For the electrophysiological recordings, a coverslip with adherent cells was transferred to a continually perfused (1 ml/min) recording chamber (RC-13, Warner Instrument, Hamden, CT).

Electrophysiological recordings. The whole cell current of Kv4.3 was recorded using a patch-clamp technique with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) at room temperature (22–24°C). The data were stored using a Digidata 1200A (Molecular Devices) acquisition board-equipped IBM-compatible computer. Currents were sampled at 5 kHz and filtered at 2 kHz (four-pole Bessel filter). Pulse generation and data acquisition were controlled using pClamp 10.0 software (Molecular Devices). Patch electrodes were fabricated using PG10165–4 glass capillary tubing (World Precision Instruments, Sarasota, FL). Liquid junction potentials between external and pipette solutions were offset. In the whole cell configuration, average series resistances were 3.9 MΩ. The effective series resistances were usually compensated by 80% when the current exceeded 1 nA. Voltage drops, based on the calculated residual series resistance, were <5 mV.

Solutions and drugs. The pipette solution contained (in mM) 140 KCl, 1 CaCl2, 1 MgCl2, 10 HEPES, and 10 EGTA, and was adjusted to pH 7.3 with KOH. The bath solution contained (in mM) 140 NaCl, 5 KCl, 1.3 CaCl2, 1 MgCl2, 20 HEPES, and 10 glucose, and was adjusted to pH 7.3 with NaOH. Genistein, tyrphostin 23, tyrphostin 25, lavendustin A, orthovanadate, daidzin, and genistein (Calbiochem, San Diego, CA) were dissolved in dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO). The concentration of DMSO in the final dilution was <0.1%, and this DMSO concentration had no effect on Kv4.3 currents.

Data analysis. Analysis of data was performed using pClamp 10.0 software (Molecular Devices) and Origin 8.0 software (Microcal Software, Northampton, MA). The concentration-response curve for Kv4.3 current inhibition by genistein was fitted to the following equation:

\[ I = I_{\text{max}} \left[ 1 + \left( \frac{[\text{drug}]}{IC_{50}} \right)^n \right] \]

where \( I \) is the normalized current inhibition, \( I_{\text{max}} \) is the maximal inhibition, and \( n \) is the Hill coefficient.

Phosphorylation-independent inhibition of Kv4.3 by genistein. Because Kv4.3 has multiple consensus sites for PTK phosphorylation, we determined whether genistein-induced inhibition of Kv4.3 was mediated through PTK inhibition by using other PTK inhibitors that are structurally and mechanistically different from genistein (tyrphostin 23, tyrphostin 25, and lavendustin A), and orthovanadate, a protein tyrosine phosphatase inhibitor (Fig. 2). Because the maximal inhibitory effect of tyrphostins was observed after 16 h of incubation (25), we tested the effects of genistein after 16 h of incubation with either tyrphostin 23 or tyrphostin 25. Preincubation with 100 μM of either tyrphostin 23 or tyrphostin 25 had no effect on the activation and inactivation kinetics of Kv4.3 currents compared with control currents (Fig. 2A). The addition of 100 μM genistein to a bath solution containing 100 μM of either tyrphostin 23 or tyrphostin 25 decreased the Kv4.3 currents by 48.76 ± 3.19 and 43.74 ± 3.33%, respectively (n = 6). A 10-min treatment with 10 μM lavendustin A did not signifi-
cantly inhibit Kv4.3 currents. Subsequent application of 100 μM genistein reduced the peak amplitude of Kv4.3 currents by 50.34 ± 3.21% (n = 6). A similar experiment with orthovan¬
date was carried out, resulting in genistein-induced inhibition of Kv4.3 by 52.57 ± 2.31% (n = 5), while exposure to 1 mM orthovanadate did not significantly inhibit Kv4.3 currents (Fig. 2B). We tested two other inactive structural analogs: genistin and daidzein (Fig. 2C). Although genistin (100 μM) did not affect Kv4.3 currents (n = 6), daidzein decreased the peak amplitude of Kv4.3 in a concentration-dependent manner (n = 6). However, complete inhibition could not be achieved, and the degree of inhibition by daidzein was less than that by genistein at each concentration examined. In addition, daidzein had no effect on the activation or inactivation kinetics of the Kv4.3 currents. Daidzein (300 μM) inhibited Kv4.3 by 41.24 ± 3.27% (n = 6). Coapplication of daidzein (300 μM) and genistin (100 μM) produced an inhibition of Kv4.3 of 53.09 ± 2.94%. This value was not significantly different from that seen in the presence of genistein alone. These results strongly suggest that genistein-induced inhibition of the Kv4.3 currents was independent of phosphorylation and dephosphorylation processes, but might have resulted from a direct interaction between genistein and the Kv4.3 channels.

Voltage-dependent inhibition of Kv4.3 by genistein. Figure 3A shows the effect of 100 μM of genistein on the current-voltage relationships for Kv4.3. Under control conditions, the Kv4.3 current was activated at pulses greater than −30 mV, and the current-voltage relationship was almost linear for depolarizing pulses between −10 and +60 mV. The inhibition of Kv4.3 currents by genistein was observed in the entire voltage range for which Kv4.3 was activated. By plotting normalized inhibition vs. potential, a high degree of inhibition was observed, with strong voltage dependence between −20 and +20 mV, which included the channel-opening voltage range (Fig. 3B). At a depolarizing potential of −20 mV, 100 μM genistein inhibited the Kv4.3 currents by 28.6 ± 1.4% (n = 6). This inhibition increased continuously to 46.8 ± 2.0% (n = 6) at +20 mV (ANOVA, F_{5,25} = 24.75, P < 0.05). However, inhibition in the voltage range between +20 and +60 mV, where the channels are fully activated, was not voltage dependent.

Effects of genistein on the activation and inactivation kinetics of Kv4.3. As shown in Fig. 1, genistein decreased the peak amplitude of Kv4.3 currents in a concentration-dependent manner, but did not alter the time course of the Kv4.3 currents during depolarization. Thus we studied the effect of genistein on the activation and inactivation kinetics of Kv4.3. Current traces were fitted to a single exponential function to estimate the rate of current activation. Under control conditions, the time constant of activation was 0.58 ± 0.02 ms (n = 9). In the presence of genistein, the time constants of activation were 0.57 ± 0.04, 0.57 ± 0.03, and 0.58 ± 0.04 ms (n = 9) for 10, 30, and 100 μM, respectively. The kinetics of activation was not significantly affected by genistein. The time course of inactivation of Kv4.3 at 40 mV was fitted to a double-exponential function under control conditions, with a fast time constant of 25.33 ± 1.46 and a slow time constant of 113.46 ± 2.25 ms (n = 9). Genistein did not affect the kinetics of Kv4.3 inactivation (fast time constants: 24.34 ± 1.41, 22.99 ± 1.51, and 23.06 ± 1.64 ms; slow time constants: 115.13 ± 2.87, 116.39 ± 2.72, and 119.13 ± 2.44 ms, n = 9, for 10, 30, and 100 μM, respectively). No significant treatment effect of the different genistein concentrations was found for either the fast or slow time constant.

Fig. 1. A: concentration dependence on the genistein-induced inhibition of voltage-de¬
dependent K⁺ channel (Kv) 4.3. Whole cell Kv4.3 currents were elicited by 500-ms step depolarization to +40 mV from a holding potential of −80 mV at 10-s intervals. The control current and currents following the addition of 10, 30, 100, and 300 μM genistein are indicated. The reduction in the peak amplitude of Kv4.3 current at +40 mV was used as an index of inhibition. B: reversible inhibi¬
tion of Kv4.3 by genistein. Whole cell cur¬
rents were elicited by 500-ms depolarizing pulses of +40 mV from a holding potential of −80 mV at 10-s intervals under control con¬
ditions and in the presence of genistein. The time course of inhibition in the presence of 100 μM genistein is shown. Maximal inhibi¬
tion occurred −1 min after drug application began. Complete recovery from inhibition was observed after washout of the drug. The steady-state amplitudes of the currents were plotted as a function of time. The bar indi¬
cates the time of genistein application. Values are means ± SE.

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Voltage dependence of the steady-state activation and inactivation of Kv4.3 by genistein. Figure 4A shows the steady-state activation curves of Kv4.3 currents under the control conditions and in the presence of genistein. The potential of the $V_{1/2}$ and the $k$ of the steady-state activation curves were $-9.17 \pm 0.03$ mV and $4.33 \pm 0.02$ mV for the control, and $-9.21 \pm 0.04$ and $4.27 \pm 0.05$ mV for 100 $\mu$M genistein ($n = 5$), respectively (Fig. 4B). Thus genistein had no effect on the voltage dependence of the steady-state activation. The steady-state inactivation curves for Kv4.3 under the control conditions had a $V_{1/2}$ of $-48.71 \pm 1.42$ mV and a $k$ of $4.52 \pm 0.24$ mV ($n = 6$) (Fig. 5A). Genistein significantly shifted the inactivation curve ($V_{1/2}$) to a hyperpolarized potential in a concentration-dependent manner ($-52.36 \pm 2.10$ mV at 30 $\mu$M and $-67.94 \pm 2.36$ mV at 100 $\mu$M; $n = 6$). However, no significant change in $k$ was observed for the curve in the presence of genistein ($4.66 \pm 0.32$ mV at 30 $\mu$M and $4.84 \pm 0.31$ mV at 100 $\mu$M; $n = 6$). Whereas the apparent dissociation constant ($K_i$) for genistein-induced inhibition of Kv4.3 in the closed state could be estimated from the reduction in the peak amplitude of Kv4.3, the apparent $K_i$ for genistein-induced inhibition in the inactivated state was estimated from the concentration-dependent shift in the steady-state inactivation curves (6). The theoretical value of $K_i$ was calculated to be $1.17 \pm 0.23$ $\mu$M ($n = 6$) (Fig. 5B).

Effect of genistein on closed-state inactivation of Kv4.3. Previous studies have shown that Kv4.3 channels are predominantly inactivated from the closed state without opening in the subthreshold voltage range (37). We tested the effect of genistein on the kinetics of closed-state inactivation. Under control conditions, a 12-s conditioning pulse to $-60$ mV inhibited $53.2 \pm 4.3\%$ ($n = 8$) of the Kv4.3 currents (Fig. 6A). The time course for closed-state inactivation was fitted to a single-exponential function with a time constant of $4.33 \pm 0.44$ s ($n = 8$). In the presence of 100 $\mu$M genistein, the rate of close-state inactivation was accelerated, and inactivation was almost complete after a 1-s conditioning pulse. Closed-state inactivation was also fitted to a single-exponential function with a time constant of $0.99 \pm 0.08$ s ($n = 8, P < 0.05$) (Fig. 6B).

Use-dependent inhibition of Kv4.3 by genistein. Figure 7A shows the current traces of the Kv4.3 obtained after applying a pulse train at a frequency of 1 or 2 Hz under control conditions or in the presence of 100 $\mu$M genistein. Under control conditions, the peak amplitude of the Kv4.3 current decreased by $2.2 \pm 0.01$ and $17.2 \pm 0.02\%$ ($n = 5$) after the application of a train of depolarizing pulses at 1 Hz, respectively (Fig. 7B). In the presence of 100 $\mu$M genistein, however, a reduction in the peak amplitude was elicited by the first depolarizing pulse, which averaged $7.2 \pm 0.5$ and $20.4 \pm 0.02\%$ ($n = 5$) inhibition at 1 and 2 Hz, respectively. Thereafter, the current amplitude was slightly affected by the train of pulses. These results suggest that there was a weak use dependence of genistein action.

**DISCUSSION**

The results of the present study can be summarized as follows: 1) genistein decreased the peak amplitude of Kv4.3 in a concentration-dependent manner without modifying current kinetics; 2) the inhibition of Kv4.3 by genistein occurred as a...
result of direct interaction between genistein and the Kv4.3 channels, that is, through a PTK-independent mechanism; and
3) genistein induced a shift in the voltage dependence of the steady-state inactivation curves of Kv4.3 to the hyperpolarizing direction in a concentration-dependent manner and significantly accelerated the closed-state inactivation.

In the present study, several lines of evidence suggest that genistein appears to have direct, PTK-independent effects on the Kv4.3 currents. The time course of genistein inhibition of Kv4.3 was very rapid, and the maximal effect was obtained within 1 min. The reversal of Kv4.3 inhibition occurred just as rapidly, suggesting that both effects might be due to nonspecific inhibition of the channels rather than to inhibition of intracellular PTK activity. Generally, the effects of phosphorylation-mediated events on ion channels occur on a time scale of several minutes (11). If genistein induced the inhibition of Kv4.3 through a PTK-dependent mechanism, then other PTK inhibitors should produce a similar effect. We examined the actions of a variety of PTK inhibitors (tyrphostin 23, tyrphostin 25, and lavendustin A), which are structurally and mechanistically different from genistein. These agents are selective and cell-permeable PTK inhibitors, and the concentrations of PTK inhibitors used in our experiments are sufficient to completely inhibit PTK activity (13, 16, 25). Our data showed that these PTK inhibitors had no effect on the genistein-inhibited inhibition of Kv4.3. Orthovanadate, which is an inhibitor of protein phosphatases, increases basal tyrosine phosphorylation and is used to antagonize the effects of PTK inhibitor. For example, orthovanadate has been shown to antagonize the inhibitory effect of genistein on L-type Ca\(^{2+}\) currents and delayed-rectifier K\(^{+}\) currents in guinea pig ventricular myocytes (26, 29). In the present study, pretreatment with orthovanadate had no effect on the inhibitory action of genistein on Kv4.3. This result indicates that the basal activity of endogenous tyrosine kinase does not play a significant role in regulating Kv4.3. However, daidzein, an inactive analog of genistein, is structurally related to genistein and showed a similar inhibitory effect.
on Kv4.3, albeit at a lower potency. Moreover, the kinetics of Kv4.3 currents remained unaffected, and the effect was completely reversed on washout. In addition, genistin, another inactive structural analog, had no effect on either the amplitude or kinetics of Kv4.3 currents. Our results confirmed that the mechanism by which genistein inhibits Kv4.3 is similar to the mechanism by which it is known to inhibit several other ion channels. Genistein inhibited voltage-dependent Na⁺ channels, whereas daidzein had moderate effects, and genistin had a weak effect (24). Bath application of genistein decreased the L-type Ca²⁺ current of rat ventricular cells in a concentration-dependent manner, and daidzein had nearly the same inhibitory effect on Ca²⁺ currents (42). These results suggest that the effect of genistein may be caused by the direct blocking of Ca²⁺ channels. Genistein inhibited delayed-rectifier K⁺ currents in guinea pig ventricular myocytes via a tyrosine kinase-independent pathway, and daidzein showed a similar effect but with a lower potency (27, 39). Interestingly, the degree of inhibition is much greater with genistein than that obtained with the same concentration of daidzein. Both genistein and daidzein have two benzene rings (A and B) as a common structural feature, but the structural difference, a hydroxyl group missing from the side chain of the A ring, is thought to be responsible for the lower potency of daidzein during ion channel inhibition. These results suggest that structure-related mechanisms and a phosphorylation-independent signal trans-

Fig. 5. Effects of genistein on the steady-state inactivation of Kv4.3. A: the currents were evoked by 1-s prepulses that were varied from −110 to 0 mV stepped by 10 mV and a 500-ms depolarizing pulse to +40 mV in the absence and presence of 30 and 100 μM genistein. Steady-state inactivation curves are shown as a plot of normalized peak currents during the depolarizing pulse as a function of the conditioning potential. The curves represent the best-fit Boltzmann equations. B: plot of exp(ΔV/k) against genistein concentrations. The V₁/₂ and k values were obtained from the steady-state inactivation curves. The concentration-dependent shift in the midpoint (ΔV) was determined as the difference between V₁/₂ values in control conditions and at 30 and 100 μM genistein (n = 6). The solid line represents the linear fit of the data: exp(ΔV/k) = 0.64 ± 0.85 [genistein], where [genistein] represents the genistein concentration. Kᵢ, the reciprocal of the slope, was calculated from this fit. Values are means ± SE. See MATERIALS AND METHODS for definitions of other terms.
duction pathway may contribute to the inhibitory action of genistein and daidzein. Because we only tested the PTK dependence of the inhibitory actions of genistein on Kv4.3, our results cannot rule out the possibility that the inhibition of Kv4.3 by genistein occurs via the nonspecific inhibition of protein kinase A and C.

One of the main findings of the present study is that genistein inhibited the Kv4.3 currents in a concentration-dependent manner without a significant effect on current kinetics on depolarization. The initial control pulse and conditioning pulse were applied from a membrane potential of −100 mV. B: the current amplitudes evoked by the second pulse, relative to the amplitude resulting from the initial control pulse, were plotted against the duration of the conditioning pulse. The data fit well to a single-exponential function. Values are means ± SE.

and of vascular smooth muscle cell K⁺ currents during depolarization, but did not change the steady-state activation of Kv1.3 (34, 35). These effects were not mediated by inhibition of tyrosine kinase activity, but might have been due to direct inhibition. The activation kinetics of Kv1.4 were profoundly slowed, and the steady-state activation curve was shifted in a depolarizing direction (43). The inactivation kinetics of Kv1.4 were slowed in the subthreshold range of depolarization, but remained unchanged at depolarizing potentials (43). Genistein produced no significant voltage-dependent changes of transient outward K⁺ current activation or inactivation (12, 38). In the present study, genistein affected neither the activation nor the inactivation kinetics of Kv4.3, but it shifted the steady-state inactivation curves in a hyperpolarizing direction. This finding can be explained by preferential binding to the channel in the inactivated state with channel inhibition. Our results indicate that the affinity of Kv4.3 for genistein is 100 times greater for the inactivated state than for the closed state. Kv4.3 channels can be inactivated from either the open or closed states, but inactivation most frequently occurs from the closed state without activation (37). Accordingly, genistein caused a marked acceleration of the closed-state inactivation of Kv4.3. A possible mechanism of action is that genistein, which is highly lipophilic, alters the mechanical properties of membranes.

Fig. 7. Effect of genistein on use-dependent inhibition of Kv4.3. A: Kv4.3 current traces obtained from applying ten 500-ms depolarizing pulses of +40 mV from a holding potential of −80 mV at 1 or 2 Hz in the absence or presence of genistein. B: plot of normalized current as a function of the number of pulses. The peak amplitudes of the current at every pulse were normalized to the peak amplitude of the current obtained as a result of the first pulse. Values are means ± SE.
thereby modulating the inactivation kinetics of Kv4.3 via an allosteric mechanism. Consistent with our results, genistein modulated gramicidin A channels in planar phospholipid bilayers by altering bilayer mechanical properties via a phosphorylation-independent mechanism, whereas daidzein had only modest effects, and genistin had no effect (18).

Genistein exists in both the monoanionic and neutral forms under physiological conditions (44). Thus genistein inhibited Kv4.3 by at least two different mechanisms: binding to the closed state and accelerating the closed-state inactivation of Kv4.3 channels. Closed states, which are predominant at hyperpolarized membrane potentials, supposedly bind genistein with low affinity, whereas closed-inactivated states bind at subthreshold levels of depolarization with high affinity. Whereas interaction of genistein with Kv4.3 channels was voltage independent in the full activation voltage range, genistein resulted in voltage-dependent inhibition of Kv4.3 over the voltage range that corresponds to channel activation. This result was attributed to the voltage dependence of Kv4.3 inactivation in the subthreshold voltage range and the preferential interaction of genistein with inactivated Kv4.3 channels. This may explain the low degree of use-dependent inhibition of Kv4.3 by genistein.

Genistein is a major bioactive isoflavone, abundant in some vegetables, that has proven to have beneficial cardiovascular effects in both animal and human studies (5). Kv4.3 is responsible for the primary transient outward K+ current in the heart (30). This channel is an important repolarizing current that determines the amplitude and duration of action potential in the heart. Inhibition of transient outward K+ currents, which are mainly mediated by atrial and ventricular Kv4.3 in humans, may contribute to prolongation of action potential duration (8). Consequently, Kv4.3 channels are a potential therapeutic target for antiarrhythmic drugs (41). The inhibitory effect of genistein on Kv4.3 may, therefore, have antiarrhythmic activity that could explain, at least in part, its cardiovascular effects. In failing hearts, however, downregulation of Kv4.3 was reported, and an excessive prolongation of action potential duration may produce proarrhythmic effects (20, 21). Plasma concentrations of genistein were relatively low and generally <40 nM in humans consuming diets devoid of soy, but were considerably higher in vegetarians (32). However, the average plasma concentration of genistein in Japanese subjects consuming a traditional diet was 0.1–0.27 μM, and plasma genistein concentrations as high as 2.4 μM were measured (1, 15). In the present study, we estimated the $K_\text{i}$ for the binding to inactivated Kv4.3 to be 1.17 μM: therefore, the effect of genistein on Kv4.3 could be clinically relevant. Thus our observation that genistein inhibits Kv4.3 provides promising information about the molecular action by which genistein exerts cardiac effect. Before extrapolating our results to clinical application, however, it should be noted that the pore-forming α-subunit of the Kv4.3 channel protein itself can form voltage-gated K+ channels in heterologous expression systems (28). Kv4.3 coassembles with a large variety of ancillary subunits in native tissues, and β-subunits are not coexpressed in our expression system. Thus it is yet to be determined whether genistein-induced inhibition of Kv4.3 occurs in native cardiomyocytes, because the interaction between these proteins can alter the drug sensitivity of Kv4.3 channels (7).

In conclusion, the results of the present study suggest that genistein directly inhibits Kv4.3 currents by binding to the closed-inactivated state of the channel. Furthermore, the inhibitory action of genistein is not mediated via an inhibition of the PTK activity. When genistein is used to study the effects of PTK phosphorylation on ion channels, therefore, the nonspecific effects of this drug should be taken into consideration, and the results must be interpreted with great caution. In addition, the present study provides a possible explanation for mechanisms that underlie some of the cardiac effects of genistein.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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