J Physiol Biochem, **65** (2), 113-124, 2009

# Genistein effects on $Ca^{2+}$ handling in human umbilical artery: inhibition of sarcoplasmic reticulum $Ca^{2+}$ release and of voltage-operated $Ca^{2+}$ channels

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(Received on October, 2008)

F. SPERONI, A. REBOLLEDO, S. SALEMME, R. ROLDÁN-PALOMO, L. RIMORINI, M.C. AÑÓN, A. SPINILLO, F. TANZI and V. MILESI. *Genistein effects on $Ca^{2+}$ handling in human umbilical artery: inhibition of sarcoplasmic reticulum $Ca^{2+}$ release and of voltage-operated $Ca^{2+}$ channels.* J Physiol Biochem, **65** (2), 113-124, 2009.

Isoflavones are a group of natural phytoestrogens including the compound genistein. Health beneficial effects have been attributed to the consumption of this compound, but the fact that it has estrogen-like activity has raised doubts regarding its potential risk in infants, newborns, or in the fetus and placenta during pregnancy. This work is aimed at studying genistein effects on $Ca^{2+}$ handling by smooth muscle cells of the human umbilical artery (HUA). Using fluorometric techniques, we found that in these cells genistein reduces the intracellular $Ca^{2+}$ peak produced by serotonin. The same result could be demonstrated in absence of extracellular $Ca^{2+}$, suggesting that the isoflavone reduces $Ca^{2+}$ release from the sarcoplasmic reticulum. Force measurement experiments strengthen these results, since genistein reduced the peak force attained by intact HUA rings stimulated by serotonin in a $Ca^{2+}$-free solution. Moreover, genistein induced the relaxation of HUA rings precontracted either with serotonin or a depolarizing high-extracellular $K^+$ solution, hinting at a reduction of extracellular $Ca^{2+}$ entry to the cell. This was confirmed by whole-cell patch-clamp experiments where it was shown that the isoflavone inhibits ionic currents through voltage-operated $Ca^{2+}$ channels. In summary, we show that genistein inhibits two mechanisms that could increase intracellular $Ca^{2+}$ in human umbilical

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smooth muscle cells, behaving in this way as a potential vasorelaxing substance of fetal vessels. Taking into account that genistein is able to cross the placental barrier, these data show that isoflavones may have important implications in the regulation of feto-maternal blood flow in pregnant women who consume soy-derived products as part of their meals.

**Key words:** Human vascular smooth muscle, Genistein, Calcium channels, Umbilical artery.

Isoflavones are a group of natural phytoestrogens including the compound genistein. This is a nonsteroidal estrogen-like compound present in significant quantities in human diet, being soy products the major dietary sources of isoflavones. This molecule exists in different forms depending on its glycosilation and processing conditions, and hydrolysis within the digestive tract can transform it and increase the aglycone fraction (10, 23). In humans, after a single soy meal the isoflavone concentration rises slowly and reaches maximum values around the micromolar range at 7-8 hours (12). The presence of genistein has been detected in plasma, urine (1, 27), human milk (9) and in prostatic tissue (3) of subjects that consume such products, as well as in amniotic fluid during the second trimester of pregnancy (8) and at birth (2). A wide spectrum of health beneficial effects have been attributed to the consumption of this natural compound, most of them related to a decrease in the risk of cardiovascular diseases and cellular proliferation (15). However, since genistein is a phytoestrogen, and hence capable of stimulating cellular estrogen receptors (4), controversy still exists regarding the effects of these compounds on human immature estrogen sensitive target tissues as well as their potential effects on the fetus due to in utero exposition. For instance, it has been reported that the placenta is a target tissue for genistein action during gestation (22), while exposure to genistein resulted in significant feminization of the male mammary glands in rats (26).

Regarding its mechanism of action, genistein is a well-known non-selective tyrosine kinase inhibitor, and there are numerous reports showing different genistein-induced cellular effects mediated by this inhibition of tyrosine kinases. There are also descriptions of other non-genomic effects of this compound related to its capability to inhibit $Ca^{2+}$ pathways directly or through hyperpolarization induced by the opening of $K^+$ channels, like some estrogen compounds do (i.e. 17-b-estradiol) (5). Particularly, genistein has shown relaxing effects mediated by a direct block of $Ca^{2+}$ channels in rabbit basilar artery (25).

Our work is focused on the effects of genistein on a fetal vessel, such as the human umbilical artery (HUA), with special attention on the mechanisms involved in the regulation of intracellular $Ca^{2+}$ concentration and force development. Using microfluorimetry, isometrical force measurements and patch-clamp techniques, we present data showing that genistein, acting on different cell structures involved in the handling of intracellular $Ca^{2+}$, is able to induce a decrease in intracellular $Ca^{2+}$ concentration and consequently produce a relaxation of this vessel. Since the isoflavones are able to cross the placental barrier (8, 22), these findings may contribute to the understanding of the

regulation of maternal-fetal blood flow in the case of pregnant women who include soy products in their diet.

## Material and Methods

Umbilical cords (n = 32) were obtained after vaginal and caesarean deliveries performed in several private clinics of La Plata, Argentina and in the Policlinico San Matteo, Pavia, Italy. They were placed in a transport solution of the following composition (in mM): 130 NaCl, 4.7 KCl, 1.17 $KH_2PO_4$, 1.16 $MgSO_4$, 24 $NaCO_3H$, 2.5 $CaCl_2$, pH 7.4 at 4 °C and immediately taken to our laboratory where they were stored at 4 °C and used before the next 24 h. All the vascular preparations were classified as surgical discard specimens and thus they were exempted from patient consent requirements.

*Intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) measurement by microfluorimetry in HUA smooth muscle cells.*– The HUAs were dissected from the Wharton´s jelly from the umbilical cord and placed in a Petri dish filled with a physiological saline solution (PSS) of the following composition (in mM): 150 NaCl, 6 KCl, 1 $MgCl_2$, 1.6 $CaCl_2$, 10 HEPES and 10 glucose. They were cleaned of adherent connective tissue, and cut in small rectangular strips. These were then incubated for 150-200 min at room temperature in PPS containing 16 µM fura-2 AM (1 mM fura-2 AM in DMSO stock solution). They were afterwards washed with PSS without fura-2 AM and fixed to the bottom of a Petri dish. *In situ* smooth muscle cells were visualized by an upright epifluorescence microscope (Zeiss, Axiolab) equipped with a 100-W Hg lamp and a Zeiss 63x Achroplan water immersion objective (0.75 numerical aperture). The cells were excited alternately at 340 or 380 nm and the emitted light was detected at 510 nm. A neutral density filter (0.3 optical density) reduced the overall intensity of the exciting light and a second neutral density filter (optical density = 0.3) was coupled to the 380 nm filter to approach the intensity of the 340 nm light. The exciting filters were mounted on a filter wheel (Lambda 10, Sutter Instrument, Novato, Calif., USA). Custom software, working in the LINUX environment, was used to drive the camera (Extended-ISIS Camera, Photonic Science, Millham, UK), the filter wheel, and to measure and plot on-line the fluorescence from about 5 rectangular regions of interest (ROI), which enclosed 1 to 5 single cells each. Variations in the $[Ca^{2+}]_i$ were monitored by evaluating for each ROI the ratio of the fluorescence signal emitted at 510 nm when exciting at 340 and 380 nm. Experiments were carried out at room temperature (20–23 °C) and ratio measurements were performed every 2.0 s.

There were two kind of protocols performed with this technique. In order to investigate genistein effects on $[Ca^{2+}]_i$ increase produced by 5-HT, we performed two successive exposures of HUA segments to 5-HT in control PSS (separated by 15 min) and in the second, genistein was also present between 20 to 180 seconds before 5-HT. A second group, designed to focus only on $Ca^{2+}$ released from intracellular stores, consisted in similar protocols as the ones described before, but performed in a "$Ca^{2+}$-free PSS" containing 1 mM EGTA but no $CaCl_2$.

*Isometric force measurements.*– HUA segments were obtained as before and then cut into 3-4 mm wide rings. The ring vessel was gently suspended between two stainless steel wires in a water-jacketed

organ bath kept at 37 °C and filled with a Krebs-bicarbonate solution (KS) of the following composition (in mM): 130 NaCl, 4.7 KCl, 1.1 $MgSO_4$, 24.0 $NaHCO_3$, 1.2 $Na_2PO_4H$, 1.6 $CaCl_2$ and 11glucose, bubbled with a mixture of 5% $CO_2$ and 95% $O_2$, giving a pH of 7.40. The lower wire was fixed to a vertical plastic rod immersed in the organ bath, while the upper one was rigidly attached to a force transducer (Letica TRI-201). The signals from the force transducers were amplified and driven into an analog-digital board (DT16EZ, Data Translation, Inc., Marlboro, MA, USA) mounted in a desktop computer. On-line recordings and files for later processing were obtained with appropriated software (Labtech Notebook Pro, Laboratory Technology Corp., Wilmington, MA, USA).

A passive force of ≈ 2 grams was applied to the preparations. After stabilization, the different experimental protocols were performed. First, to investigate genistein effects on $Ca^{2+}$ release from intracellular stores, we stimulated arterial rings with 1 µM 5-HT, with or without preincubation with genistein (20 µM), in a $Ca^{2+}$-free KS of the following composition (in mM): 130 NaCl, 4.7 KCl, 1.1 $MgSO_4$, 24.0 $HCO_3Na$, 1.2 $PO_4HNa_2$, 1.0 EGTA and 6 glucose, bubbled with a mixture of 5% $CO_2$ and 95% $O_2$, giving a pH of 7.40. Afterwards, to investigate genistein actions on extracellular $Ca^{2+}$ entry, the isoflavone was added on top of stable contractions produced by stimulating arterial rings with 5-HT or by depolarization induced with a high $K^+$ KS prepared by rising the KCl of the KS to 80 mM and lowering the NaCl accordingly in order to maintain osmolarity.

Since genistein was dissolved in DMSO, appropriate amounts of this solvent (usually no more than 20 µl in a 20 ml final volume) were added to control experiments. Our previous experience shows, however, that these concentrations of DMSO do not affect the experimental results.

*Patch-clamp recordings.*– The arteries were dissected from the Wharton's jelly just before the cell isolation procedure. HUA smooth muscle cells were obtained by a method based on the one described by Klockner (13) and later modified in our laboratory (21) in order to diminish the enzyme content in the dissociation medium (DM). Briefly, segments of HUA were cleaned of any residual connective tissue, cut in small strips and placed for 15 minutes in a DM containing (in mM): 140 NaCl, 5 $KH_2PO_4$, 5 $MgCl_2$, 20 glucose, 5 HEPES, pH was adjusted to 7.4 with NaOH. The strips were then placed in DM with 2 mg/ml collagenase type I during 25 minutes, with gentle agitation, at 35 °C. After the incubation period the strips were washed with DM and single HUA smooth muscle cells were obtained by a gentle dispersion of the treated tissue using a Pasteur pipette. The remaining tissue and the supernatant containing isolated cells were stored at room temperature (20–23 °C) until used. HUA smooth muscle cells were allowed to settle onto the coverglass bottom of a 3 ml experimental chamber. The cells were observed with a mechanically stabilized, inverted microscope (Zeiss-Telaval3) equipped with a 40X objective lens. The chamber was perfused for 15 minutes, at 1 $ml.min^{-1}$ by gravity, with the extracellular saline solution (ESS, see composition later) before the patch-clamp experiment was started. Application of test solutions was performed through a multibarreled pipette positioned close to the cell investigated.

After each experiment on a single cell, the experimental chamber was replaced by another one containing a new sample of cells. Only well-relaxed, spindle-shaped smooth muscle cells were used for electrophysiological recordings. Data were collected within 4-6 hours after cell isolation. All experiments were performed at room temperature (20–23 °C).

The standard tight-seal whole-cell configuration of the patch-clamp technique (11) was used to record ionic currents. Glass pipettes were drawn from WPI PG52165-4 glass on a two-stage vertical micropipette puller (PP-83, Narishige Scientific Instrument Laboratories, Tokyo, Japan) and pipette resistance ranged from 2 to 4 MΩ measured in ESS. Ionic currents were measured with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Whole-cell currents were filtered at 2 kHz, digitized (Digidata 1200 Axon Instruments, Foster City, CA) at a sample frequency of 100 kHz, and stored on a computer hard disk for later analysis. Whole-cell capacitance was compensated electronically in most cells. First, whole-cell current was controlled applying test voltage steps during a short stabilization period discarding those in which the current amplitude did not remain constant with time. After this, whole-cell $Ca^{2+}$ currents were evoked by a voltage-clamp protocol applying 10 mV increase voltage-steps from -60 to 50 mV from a holding potential of -80 mV.

*Solutions for patch clamp recording of whole cell $Ca^{2+}$ currents.*– The extracellular standard solution (ESS) contained (in mM): 116 NaCl, 4.7 KCl, 5 $CaCl_2$, 6 glucose, 5 HEPES, 10 tetraethylammonium (TEA); pH was adjusted to 7.4 with NaOH. The composition of the intracellular pipette solution (IPS) was (in mM): 130 CsCl, 5 $Na_2ATP$, 1 $MgCl_2$, 10 glucose, 10 EGTA, 20 HEPES; pH was adjusted to 7.3 with CsOH.

*Reagents.*– Genistein, serotonin (5-HT), ethylene glycol-bis(β-aminoethyl ether) N,N,N',N',-tetraacetic acid (EGTA), CsCl, $Na_2ATP$, tetraethylammonium, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), CsOH, dimethyl sulfoxide (DMSO), and collagenase type I used for cell isolation were purchased from Sigma Chemical Company (St Louis, MS, USA). Fura-2 AM was obtained from Molecular Probes (Eugene, OR, USA). All other reagents were of analytical grade and purchased from local vendors.

*Statistics.*– The results are expressed as means ± SEM. Paired or unpaired Student's t-tests were used to establish statistically significant differences between two groups. ANOVA followed by Tukey's test was used to compare multiple groups. The level of statistically significant differences was set to $p<0.05$.

## Results

*Genistein effects on $Ca^{2+}$ release from intracellular stores.*– We used serotonin (5-HT) as a vasoactive agent which in HUA is able to induce both $Ca^{2+}$ release from the sarcoplasmic reticulum and extracellular $Ca^{2+}$ entry (7). The response of the artery to 1 μM 5-HT showed two phases: the first one was an acute increase in $[Ca^{2+}]_i$ (10.25 ± 1.16 seconds in arriving to the maximum, n=6), followed by a second phase during which $[Ca^{2+}]_i$ oscillated around values greater than the resting level (Fig. 1A). After the removal of 5-HT, oscillations exhibiting a variable frequency and amplitude were still

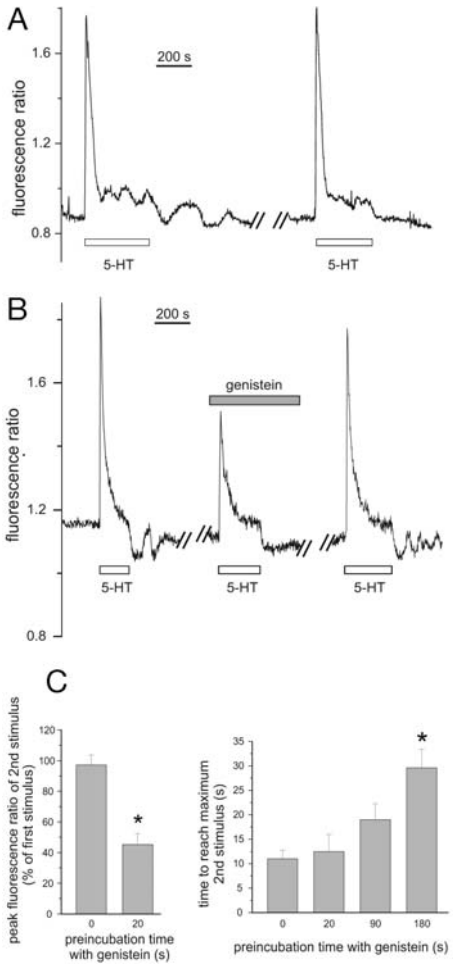


Fig. 1A. *Typical recordings showing $[Ca^{2+}]_i$ changes (measured as fura fluorescence ratio changes) induced by 5-HT in* in situ *HUA smooth muscle cells. A second identical response, separated by 15 min from the first one, is shown in the right panel. Average values for the response are given in the text. B. Typical recordings of fura fluorescence ratio changes induced by 3 applications of 5-HT separated by 15 min. The second one was performed in the presence of genistein (added 20-180 s before 5-HT). C. Average peak values of the second response shown in B expressed as % of the first response, and average time needed to reach this peak. Each bar represents a different genistein preincubation time (n = 4-5 for each preincubation time).*

The * symbol denotes statistically significant differences from 0 min.

observed during 10-12 minutes. A second application of 1 µM 5-HT after 15 min of washout with physiological saline solution (PSS) induced a $[Ca^{2+}]_i$ increase which was quite similar to the first one (see Fig. 1A for experimental records and Fig. 1C for average values of 2nd exposure to 5-HT compared with the 1st). Preincubation with 20 µM genistein for different times (between 20 to 180 seconds) reduced the initial peak $[Ca^{2+}]_i$ increase induced by a second addition of 5-HT compared to the first one (Fig. 1B and C). The time required to reach the maximum of this peak was also augmented (Fig. 1C). Both effects were reversible: after 6 minutes of genistein wash, the recovery of the peak force was of 80.3 ± 8.7% (n=7) and after 15 minutes it was of 100% (n=5).

In order to distinguish the $[Ca^{2+}]_i$ change due to the release from intracellular stores from that produced by external $Ca^{2+}$ experiments with 5-HT were now performed in a $Ca^{2+}$-free PSS (no $CaCl_2$ and addition of 1 mM EGTA). The removal of extracellular $Ca^{2+}$ immediately induced a decrease of basal $[Ca^{2+}]_i$, as was already reported by our laboratory for this vessel (20) (Fig. 2A). In this situation application of 5-HT produced a fast initial increase of $[Ca^{2+}]_i$, while the second phase of the response which was observed in the presence of external $Ca^{2+}$, was now reduced to a minimum (Fig. 2A). The magnitude of the $[Ca^{2+}]_i$ increase induced by 5-HT in $Ca^{2+}$-free PSS was not different from that obtained in the same arterial segments 15 minutes after restituting extracellular $Ca^{2+}$. Taken together, these results suggest that this first phase of $[Ca^{2+}]_i$ increase is dependent on the release from internal $Ca^{2+}$ source, likely the sarcoplasmic reticulum. Preincubation with 20 µM genistein (90 s) between the two 5-HT exposures reduced the ampli-

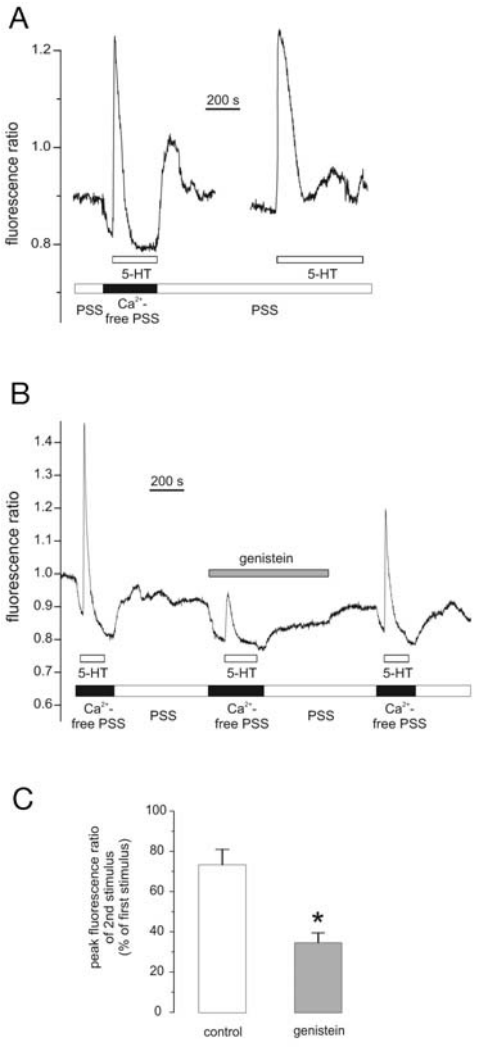


Fig. 2A. *Left panel: Typical recording of a similar experiment to the one shown in figure 1A but performed in a $Ca^{2+}$-free PSS. A second application of 5-HT after 15 min of restitution of extracellular $Ca^{2+}$ is shown in the right panel. B. Typical tracing of a protocol similar to the one shown in figure 1B where 5-HT applications were performed in a $Ca^{2+}$-free PSS. The second response was obtained in the presence of genistein (added 90 s before 5-HT). C. Average values for the peak of the second response of experiments shown in B (in % of peak of the first* response). The * symbol denotes statistically significant differences of genistein group (n=5) from controls (n=4).

tude of $[Ca^{2+}]_i$ peak produced by the second 5-HT stimulation (Figs. 2B and C). These results are indicative that genistein is able to inhibit the intracellular $Ca^{2+}$ release produced by 5-HT.

Isometric force measurements performed in HUA rings (mechanical experiments) strengthen the facts observed measuring intracellular $Ca^{2+}$ concentration. Calcium removal induced a decrease in force already described for this vessel by our laboratory (20). Contractions elicited by 1 μM 5-HT in this $Ca^{2+}$-free KS after a 20 min preincubation with genistein (20 μM) were significantly lower compared to the controls (Fig. 3A and B). Since in these conditions no extracellular $Ca^{2+}$ was present, the contractions were entirely dependent on intracellular $Ca^{2+}$ store release.

*Effect of genistein on ionic channels involved in regulating extracellular $Ca^{2+}$ entry pathways in HUA segments.–* When genistein was added acutely on top of the stable force developed by 5-HT it produced a relaxation in a concentration-dependent manner (Figs. 3C and D). Considering this, we tried the isoflavone effects on contractions induced by a different mechanism. A maximum concentration of genistein (100 μM) was added on top of contractions induced by high $K^+$ KS, where it also produced a relaxation of developed force (Figs. 3E and F).

These results indicate that genistein may be blocking one or more calcium entry pathways in these cells. First, the fact that genistein partially relaxed high $K^+$ solution-induced contractions suggests that it could be acting through its known effect of blocking voltage-operated calcium channels (VOCCs), a mechanism that could also be involved in the relaxing effect of 5-HT contractions.

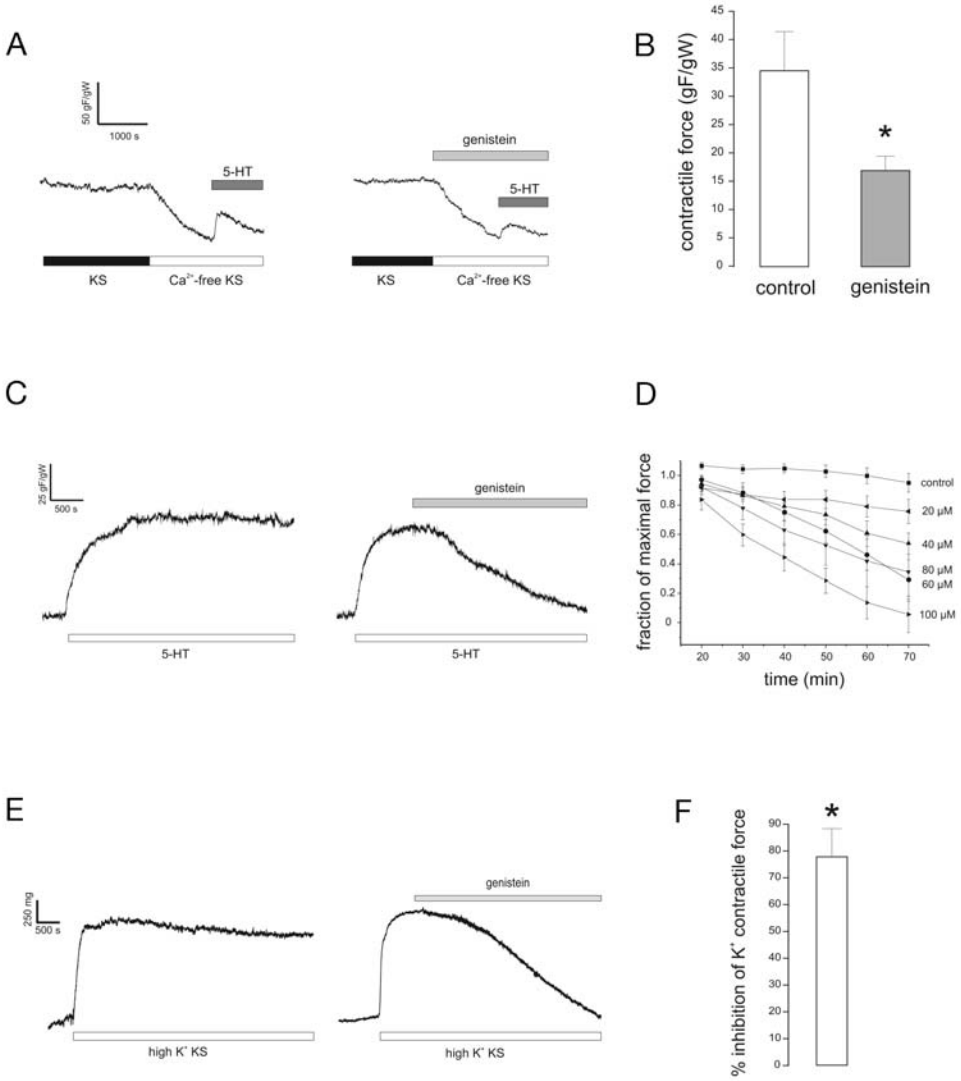


Fig. 3A. *Typical recordings of 5-HT-induced isometric contractile force of isolated HUA rings in $Ca^{2+}$-free KS, with (n=14) or without (n=7) genistein, as indicated by the bars. B. Average values of peak contractile force for experiments shown in A. The symbol * indicates statistically significant differences from control group. C. Typical recordings of the effect of genistein addition on top of stable 5-HT-induced isometric contractile force of isolated HUA rings. D. Average values of the relaxant effect induced by different doses of genistein (measured at different times) added on top of 5-HT-induced contractions as shown in C (n=5 for 20, 40 and 60 µM; n=6 for 80 µM; and n=7 for 100 µM). E. Typical recordings of the effect of genistein addition on top of stable high $K^+$ KS-induced isometric contractile force of isolated HUA rings. F. Average value of the relaxant effect of genistein added on top of stable high $K^+$ KS-induced contractions as shown in E expressed in % of the maximum contractile force (n=7).*

The symbol * indicates statistically significant differences from zero.

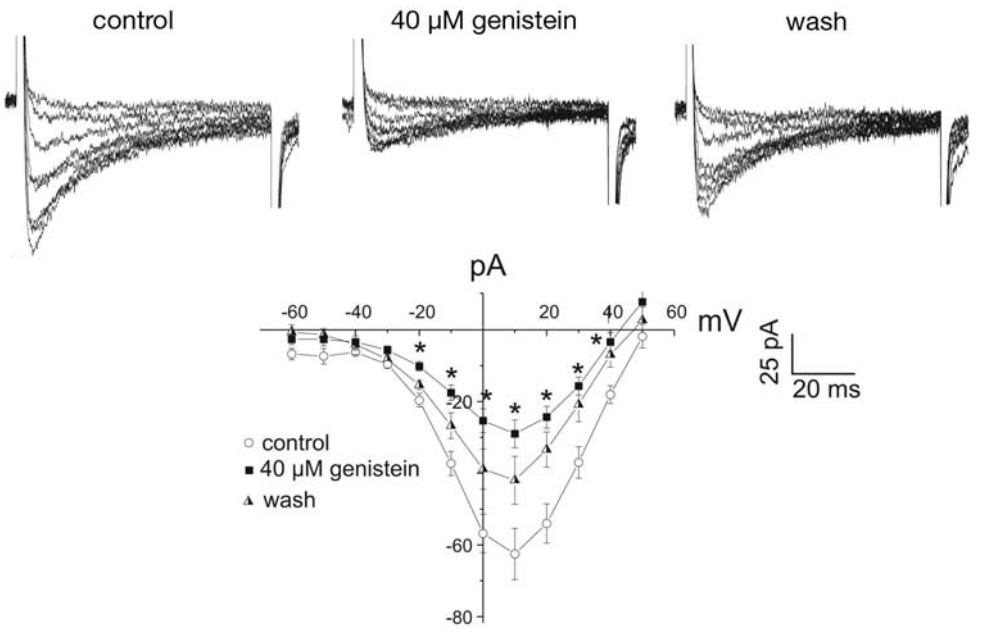


Fig. 4A. *Typical recordings of whole-cell $Ca^{2+}$ currents in isolated HUA smooth muscle cells evoked by a voltage-clamp protocol of voltage-steps from -60 to 50 mV (separated by 10 mV increments) from a holding potential of -80 mV. Left panel: control; middle panel: after 6 min of genistein; right panel: partial wash of genistein effects. B. Average current-voltage curves for experiments shown in A.*
The * symbol denotes statistically significant differences from controls (n=8, $p<0.01$).

We have already described in a previous work the characteristics of voltage-operated $Ca^{2+}$ currents in freshly dispersed HUA cells (21) using patch-clamp technique. So, in this study, we tested the effect of genistein on this current. A typical current-voltage (I-V) curve can be seen in Fig. 4. The peak amplitudes of $Ca^{2+}$ currents evoked by voltage steps between -20 and +40 mV were significantly reduced after a 6 min treatment with 40 µM genistein. The $Ca^{2+}$ current could be partially recovered by washing with control extracellular solution.

## Discussion

In this work we found that in HUA, genistein has two important target cell structures involved in the regulation of intracellular $Ca^{2+}$ concentration. Its global effect is directed to inhibit cytosolic $Ca^{2+}$ increase and extracellular $Ca^{2+}$ entry, hence diminishing vessel contraction.

Our first set of results point to genistein inhibition of $Ca^{2+}$ release from intracellular stores. Genistein attenuated the peak $[Ca^{2+}]_i$ increase produced by 5-HT and, since these effects were still observed in a $Ca^{2+}$-free solution, this implies that the isoflavone is inhibiting $Ca^{2+}$ release from intracellular reservoirs. In accordance with these results, preincubation with genistein in a $Ca^{2+}$-free solution attenuated the peak force developed by 5-HT, which in this case is solely attributed to $Ca^{2+}$ release from the sarcoplasmic reticulum. In the same line of evidence, we

have previously reported that genistein exhibits comparable effects on 5-HT contractions in rat aorta (24), and other authors found that in rabbit aorta genistein diminishes the sarcoplasmic reticulum-dependent phase of noradrenaline contractions (14), while the same occurs with 5-HT transients in culture cells derived from rat aorta (19).

However, other explanations should also be considered in order to deepen in the mechanism of such effect. For instance, genistein has been shown to activate a mitochondrial $Ca^{2+}$ uniporter (18). Alternatively, since the plasma membrane $Ca^{2+}$-ATPase is inhibited by tyrosine kinases (6), genistein treatment may enhance the pump activity. Both these effects should contribute to a decrease in the magnitude of the $[Ca^{2+}]_i$ peak produced by $Ca^{2+}$ release from the sarcoplasmic reticulum after a 5-HT stimulus.

We also present data which show that genistein reduces extracellular $Ca^{2+}$ entry to HUA smooth muscle cells, by direct action on $Ca^{2+}$ channels. The patch-clamp data we present may explain, at least in part, the relaxant effect the isoflavone had on 5-HT and KCl induced contractions since voltage operated $Ca^{2+}$ channels are involved in the sustained phase of both contraction. Hence, our data are in accordance with those of LIU *et al.* which show that in rat portal vein, genistein inhibits voltage-activated $Ca^{2+}$ channels both in the whole-cell configuration (16) and at the single channel level (17). However, most of the data on genistein effects on $Ca^{2+}$ channels involve its effects on L- and T-type channels. In the human umbilical artery, in particular, we have described the presence of P/Q-type $Ca^{2+}$ channels (21), which are not traditionally associated with vascular smooth muscle, so further experiments in this tissue should be aimed at investigating if genistein affects this subtype of channels in a different manner compared with other type of $Ca^{2+}$ channels.

In summary, we show that genistein inhibits two mechanisms that could increase intracellular $Ca^{2+}$ in human umbilical smooth muscle cells, having in this way a potential effect as vasorelaxing substance of fetal vessels. Taking into account that genistein is able to cross the placental barrier (8, 22), these data show that isoflavones may have important implications in the regulation of feto-maternal blood flow in pregnant women who consume soy-derived products as part of their meals.

*Acknowledgements*

The authors wish to thank Ms. V. Savietto for her excellent technical assistance, and Mr. P. Urdampilleta and the staff of the Instituto Central de Medicina for the provision of umbilical cords. This work was financially supported by the grants PIP 2854 from the CONICET and PICT 14455 from the ANPCYT.

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Las isoflavonas son un grupo de fitoestrógenos naturales que incluyen la genisteína. Al consumo de este compuesto se le han atribuido efectos beneficiosos para la salud, pero su actividad similar a los estrógenos permite pensar en efectos indeseados en niños o en el feto o la placenta durante el embarazo. En este trabajo se estudian los efectos de la genisteína sobre el manejo de $Ca^{2+}$ por las células de músculo liso de la arteria umbilical humana (AUH). Mediante la utilización de técnicas fluorométricas se observó que la genisteína reduce el pico de $Ca^{2+}$ intracelular producido por la

serotonina en estas células incluso en ausencia de $Ca^{2+}$ extracelular, lo que sugiere que la isoflavona reduce la liberación de $Ca^{2+}$ a partir del retículo sarcoplásmico. Los experimentos de medida de fuerza refuerzan estos resultados, ya que la genisteína redujo la fuerza pico desarrollada por serotonina en anillos intactos de AUH en una solución libre de $Ca^{2+}$. Además, la genisteína indujo la relajación de anillos de AUH precontraídos con serotonina o con una solución despolarizante de alto $K^+$ extracelular, lo que apunta a una reducción de la entrada de $Ca^{2+}$ desde el exterior de la célula. Con la técnica de "patch-clamp" en configuración de célula entera, los resultados confirmaron que la isoflavona inhibe corrientes iónicas a través de canales de $Ca^{2+}$ operados por el voltaje. En resumen, mostramos que la genisteína inhibe dos mecanismos que incrementan el $Ca^{2+}$ intracelular en células de músculo liso de AUH, comportándose de esta manera como un potencial vasorrelajante de los vasos fetales. Dado que la genisteína atraviesa la barrera placentaria, estos datos muestran que las isoflavonas podrían tener consecuencias en la regulación del flujo materno-fetal en mujeres embarazadas que incluyan productos derivados de la soja como parte de sus dietas.

**Palabras clave:** Músculo liso vascular humano, Genisteína, Canales de calcio, Arteria umbilical.

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