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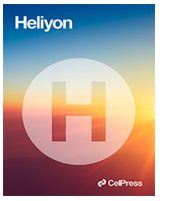

Vasodilation promoted by (*E*,*E*)-farnesol involving ion channels in human umbilical arteries

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ABSTRACT

Background: (*E*,*E*)-farnesol is a sesquiterpene alcohol derived from plants and animals that exhibits pharmacological properties in the cardiovascular system. However, its effects on human umbilical vessels remain unknown.
Purpose: Thus, this study aims to characterize the vasodilatory effect of (*E*,*E*)-farnesol in human umbilical arteries (HUA).
Study design: The tissue is obtained from pregnant women over 18 years of age, normotensive, and without prepartum complications. After collected, the tissue was segmented and dissected to remove Wharton's jelly and obtain the umbilical arteries segments.
Methods: HUA segments were isolated and sectioned into rings that were subjected to isometric tension recordings in an organ bath.
Results: (*E*,*E*)-farnesol (1 μmol/L to 1 mmol/L) promoted vasodilatory effect in HUA preparations, affecting basal tone, and inhibiting the electromechanical coupling induced by KCl 60 mmol/L with greater potency (EC_{50} 225.3 μmol/L) than the pharmacomechanical coupling induced by 5-HT 10 μmol/L (EC_{50} 363.5 μmol/L). In the absence of extracellular calcium, pharmacomechanical coupling was also abolished, and contractions induced by $CaCl_2$ or $BaCl_2$ were attenuated by (*E*,*E*)-farnesol indicating a possible direct inhibition of L-type VOCC as a mechanism of the vasodilatory effect. The vasodilator efficacy of (*E*,*E*)-farnesol on reduction of vasocontraction induced by the presence of tetraethylammonium (1 or 10 mmol/L), 4-aminopyridine (1 mmol/L) and

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glibenclamide (10 μmol/L) suggesting a possible influence of different potassium channels (BK_{Ca}, K_V and K_{ATP}).
Conclusion: These results suggest that (*E*,*E*)-farnesol may be a promising pharmacological candidate for obstetric hypertensive disorders.

Abbreviations

4-AP	4-aminopyridine
A7r5 cells	rat aortic vascular smooth muscle cell line
AC	adenylyl cyclase
$BaCl_2$	barium chloride
BK_{Ca}	large conductance Ca^{2+}-activated K^+ channels
Ca^{2+}	calcium ion
$CaCl_2$	calcium chloride
cAMP	cyclic adenosine monophosphate
(*E*,*E*)-FAR	(2*E*,6*E*)-farnesol
EC_{50}	median effective concentration
E_{MAX}	maximum effect
FPP	farnesyl pyrophosphate
GLI	glibenclamide
GPP	geranylgeranyl pyrophosphate
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase
HUA	human umbilical arteries
HVA	P-/Q-types of the high voltage-activated family
IP_3	inositol 1,4,5-trisphosphate
IUPAC	International Union of Pure and Applied Chemistry
K^+	potassium ion
K_{ATP}	ATP-dependent K+ channels
KCl	potassium chloride
K_{IR}	inward rectifier K^+ channels
LD_{50}	medial lethal dose
L-type VOCC	L-type voltage-operated Ca^{2+} channels
LVA	T-type of the low voltage-activated family
MEP	plastidial methylerythritol phosphate
MEV	cytosolic mevalonate
PLC	phospholipase C
ROCK pathway	rho-associated protein kinase (ROCK)
TEA	tetraethylammonium compound
Y-27	Y-27632 (a ROCK inhibitor)

1. Introduction

The current state of the art of science allows us to infer that the chemical-biological diversity of natural products, especially plant secondary metabolites, is an extensive source of raw material for the discovery of new pharmacological options. Terpenes/terpenoids are classic examples of this situation, whose biosynthesis in plants derives from common isoprenoid diphosphate precursors (C_5) via the plastidial methylerythritol phosphate (MEP) and/or cytosolic mevalonate (MEV) pathways [1].

In this context, farnesol (IUPAC: 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol), an acyclic sesquiterpene alcohol (C_{15}) of phytochemical occurrence present in many essential oils of medicinal plants, such as citronella, neroli, lemon grass and rose [2], is a ubiquitous molecule in nature, and is also an endogenous byproduct (and modulator) of the MEV/cholesterol biosynthetic pathway in mammalian cells, and three possible sources have been previously described for these: (1) interconversion with farnesyl pyrophosphate (FPP); (2) degradation of prenylated proteins; (3) dietary or pharmaceutical [3–6].

The literature describes that farnesol has applications in the food, cosmetic and perfumery industries, also presenting different pharmacological properties, including in the cardiovascular system. Moreover, it presents low toxicity (mean lethal dose (LD_{50}) $\geq$ 5000 mg/kg) *in vivo* [7], arousing a greater incentive to prospect its bioactivities in *ex vivo* models.

The isomeric form (2*E*,6*E*)-farnesol ((*E*,*E*)-FAR) has already been identified as one of the major constituents of plant essential oil with proven vasodilatory effect on mouse aortic arteries [8]. Studies report its effect: (1) *in vitro* to reduce Ca^{2+} signaling critical for the

contractile process of vascular smooth muscle cells; (2) *ex vivo* in antagonizing electro- and pharmacomechanical contractions in human (resistance) and non-human vessels, supporting the idea that (*E*,*E*)-FAR acts as an endogenous regulator of vascular tone; (3) *in vivo* as a hypotensive agent in rodents [9–13].

Given the above, we hypothesized that (*E*,*E*)-FAR could also act as a vasorelaxant agent in human umbilical arteries (HUA). Such a finding has impactful physiopharmacological and therapeutic implications, considering the anatomo-physiological particularities of these vessels, and the serious consequences that can occur to the fetus when blood flow is impaired, for example, in the case of gestational hypertensive disorders [14], since the physiological role of the HUA is to transport blood to the placenta to be reoxygenated [15].

Moreover, researches evaluating the action of natural products on the smooth muscle of these vessels are scarce worldwide [16]. Thus, this study aims to characterize, for the first time, the vasodilatory effect of (*E*,*E*)-FAR on healthy women's HUA.

2. Materials and methods

2.1. Drugs, gases, reagents, salts and solutions

All salts, drugs and reagents are of analytical purity, and were obtained from Sigma-Aldrich Corporation (St. Louis, Missouri, USA), Merck (Darmstadt, Germany) and Reagens (Rio de Janeiro, RJ, Brazil), and stored according to the manufacturer's instructions. The Krebs-Henseleit solution used in the experiments followed the composition (in mmol/L) provided in Dantas et al. [16]: KCl (4.8), NaCl (125), $MgSO_4$ (1.2), $CaCl_2$ (1), glucose (11), KH_2PO_4 (1.2), $NaHCO_3$ (25) and EDTA (0.3). This solution was also modified for transport/storage with the addition of HEPES (25), and for certain experimental evaluations, a Krebs-Henseleit solution with 60 mmol/L was prepared by equimolar substitution of KCl and NaCl. A Krebs-Henseleit solution with $CaCl_2$ omission was required for evaluations in Ca^{2+}-free medium, all Krebs-Henseleit types were maintained at 37 °C, pH 7.4 and O_2/CO_2 95:5.

(*E*,*E*)-FAR (CAS registration 106-28-5) was solubilized in 3% tween and diluted in Krebs-Henseleit in stock solutions (1 mol/L, 100 mmol/L, and 10 mmol/L) renewed weekly and homogenized on a vortex-type shaker before use. The other substances, 5-HT, TEA, GLI, 4-AP, $CaCl_2$ and $BaCl_2$ were diluted in distilled water, except nifedipine diluted in ethanol, and stored in stock solutions at 0–4 °C.

2.2. Tissue collection and preparation

After approval by the Human Research Ethics Committee from the Regional University of Cariri (Comitê de Ética em Pesquisa Humana da Universidade Regional do Cariri, Crato, Ceará, Brazil, number approbation 3.832.881), and by the Ethics Committee of the Maternity (Hospital e Maternidade São Francisco de Assis, Crato, Ceará, Brazil), segments of umbilical cords (10–15 cm) without structural alterations, in their distal portion - that is, close to the baby - were obtained from pregnant women over 18 years of age, normotensive and without prepartum complications, from term delivery (vaginal or cesarean) with informed consent.

In the laboratory, the segments were dissected to remove Wharton's jelly and obtain the umbilical arteries, which were then sectioned into rings (3–5 mm). The rings had their vascular endothelium mechanically removed as previously described [16,17] and were immediately suspended in glass chambers (10 mL) of organ bath equipment filled with Krebs-Henseleit under the ideal conditions cited previously, to record the isometric tension of the rings by force transducers (MLT0420, ADInstruments Bridge Amps, ADInstruments, Sydney, Australia).

The arterial rings were artificially tensioned at 3 gf for 90 min, during which time the Krebs-Henseleit solution was renewed every 15 min to avoid interference from metabolites [16]. After stabilization, the rings were qualified as viable for functional protocols when they assumed a contractile response ≥1 gf after stimulation with 60 mmol/L KCl (K60), otherwise they were discarded. Viable rings were washed with Krebs-Henseleit and equilibrated again for 15 min until return to baseline for start of experiments.

2.3. Ex vivo vascular contractility experiments

Initially, the effect of (*E*,*E*)-FAR on resting tension (basal tone) of HUA rings was investigated from increasing and cumulative increments on a concentration scale ranging from 1 μmol/L to 1 mmol/L of (*E*,*E*)-FAR to allow the construction of concentration-response curves. To evaluate the reversal of the effect of (*E*,*E*)-FAR in HUA, after exposure to the concentration-response curve at basal tone, washes every 15 min were conducted over a period of 60 min to remove (*E*,*E*)-FAR, and a contraction with depolarizing solution K60 was performed, and compared with the contraction induced by this same agent in the tissue viability period. Arterial preparations not exposed to (*E*,*E*)-FAR were used as controls.

To evaluate the effect of (*E*,*E*)-FAR on electro- and pharmacomechanical couplings, contractions were evoked by K60 and 5-HT (10 μmol/L), respectively. Upon reaching the maximal contractile response (12 ± 2 min), (*E*,*E*)-FAR (1 μmol/L to 1 mmol/L) was added cumulatively at 10 ± 5 min intervals, sufficient time for a steady-state effect to be achieved at each new concentration of (*E*,*E*)-FAR [17]. The vasodilator effect of the two most effective concentrations in these protocols (800 μmol/L and 1 mmol/L) was also evaluated in Ca^{2+}-free Krebs-Henseleit solution after K60- or 5-HT-induced contractions. Arterial preparations not exposed to (*E*,*E*)-FAR were used as controls. To assess the influence of (*E*,*E*)-FAR on the ROCK pathway, its relaxant effect on pharmacomechanical coupling in Ca^{2+}-free medium was compared to the effect of the ROCK inhibitor, Y-27632 (Y-27; 10 μmol/L).

The general participation of Ca^{2+} channels or specific L-type VOCC in the vasodilator effect of (*E*,*E*)-FAR, was assessed in Ca^{2+}-free Krebs-Henseleit solution based on methods described previously [17,18]. Briefly, HUA rings were stimulated with K60 for 15 min and then pre-incubated for 25 ± 5 min with (*E*,*E*)-FAR (800 μmol/L or 1 mmol/L) or 10 μmol/L nifedipine (positive control), and then

cumulative contractions were induced by $CaCl_2$ or $BaCl_2$ (0.1–20 mmol/L) at 5-min intervals. Arterial preparations not exposed to (*E*,*E*)-FAR or nifedipine were used as controls.

The involvement of K^+ channels in the vasodilator effect of (*E*,*E*)-FAR was verified after pre-contraction by 5-HT (10 μmol/L) for 15 min followed by pre-incubation (25 ± 5 min) of HUA rings with each of the following blockers at concentrations standardized for umbilical vessel rings [19,20], nonselective TEA (10 mmol/L), TEA (1 mmol/L) for BK_{Ca} channels and K_V channels, GLI (10 μmol/L) for K_{ATP} channels, 4-AP (1 mmol/L) for K_V channels, or $BaCl_2$ (500 μmol/L) for K_{IR} channels. Subsequently, concentration-response curves were obtained with (*E*,*E*)-FAR (1 μmol/L to 1 mmol/L). The effect was compared to the effect in arterial preparations exposed to 5-HT (10 μmol/L) alone or to (*E*,*E*)-FAR with 5-HT.

2.4. Analysis and presentation of the data

The results distinguish for each protocol, the maximum number of biological samples (*n*) used in each experiment, i.e. umbilical cord from different donors. Data are expressed as mean values of isometric tension (in gf or %) ± standard error of the mean (S.E.M.). For the concentration-response curve experiments, median effective concentrations (EC_{50}) and maximum effects (E_{MAX}) were calculated. Student's t-test, Mann-Whitney *U* test or two-way analysis of variance (ANOVA two-way) followed by Holm-Sidak test were performed where appropriate. Results were considered statistically significant when $p < 0.05$. Graphs and statistical analyses were conducted in software SigmaPlot version 12.

3. Results

3.1. Effect of (E,E)-farnesol on basal tone of human umbilical arteries

(*E*,*E*)-FAR (1 μmol/L to 1 mmol/L) promoted relaxation in the basal tone of HUA from the concentration of 100 μmol/L ($p < 0.036$), with a maximum effect (E_{MAX}) reduction of 0.39 gf and a median effective concentration (EC_{50}) 279.1 μmol/L (Fig. 1A). After incubation with the range of (*E*,*E*)-FAR concentrations the contractile response was reduced ($p < 0.001$), but the relaxing effect on basal tone was partially reversed ($p < 0.001$) after removal of (*E*,*E*)-FAR from the extracellular solution (Fig. 1B and C).

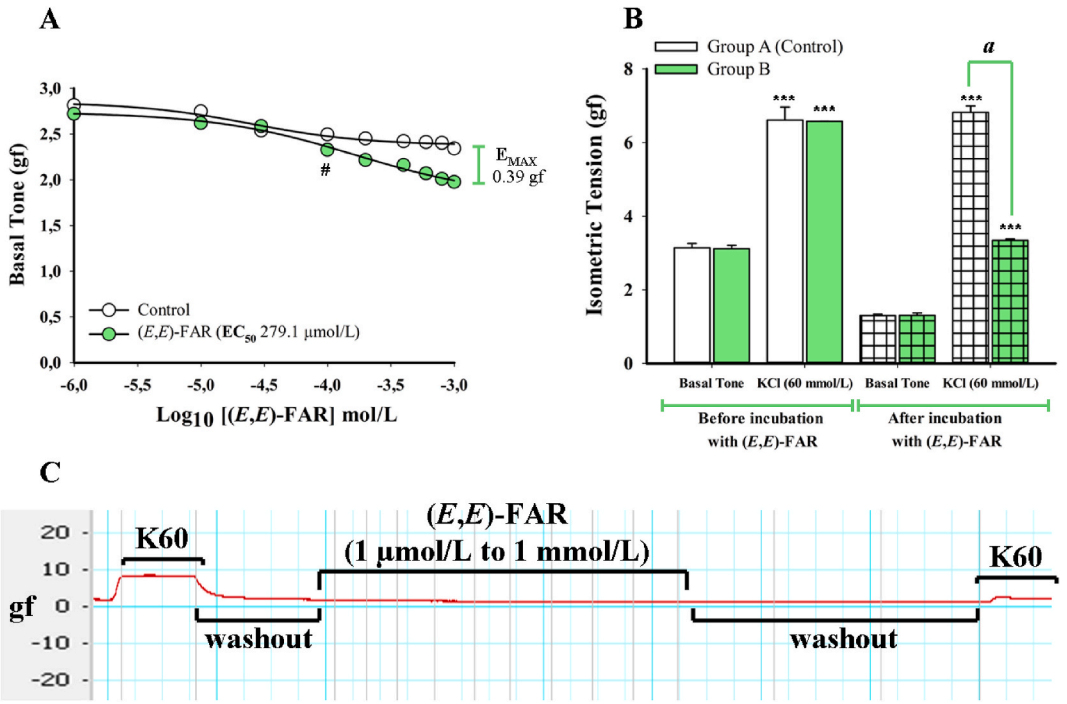

Fig. 1. Effect of (*E*,*E*)-FAR on basal tone of human umbilical arteries. (**A**) Concentration-response curve of (*E*,*E*)-FAR (1 μmol/L to 1 mmol/L) on basal tone of human umbilical arteries. (**B**) Contractile response to KCl (60 mmol/L) (K60) before and after incubation with (*E*,*E*)-FAR (1 μmol/L to 1 mmol/L), where sample group A represents the human umbilical artery rings used as control and sample group B represents the human umbilical artery rings that were incubated with (*E*,*E*)-FAR. (**C**) Original record showing the reversal of the effect of (*E*,*E*)-FAR (1 μmol/L to 1 mmol/L) on basal tone of human umbilical arteries. Values expressed as mean ± S.E.M. ($n = 5$). The # symbol indicates that from this concentration the relaxation was significant ($p < 0.05$ *versus* control in two-way ANOVA followed by Holm-Sidak). The *** or *a* symbols indicate statistical significance ($p < 0.001$ basal tone *versus* KCl or $p < 0.001$ KCl from group A *versus* KCl from group B in Student's t-test or Mann-Whitney *U* test). The value of the median effective concentration (EC_{50}) is expressed in μmol/L and the maximum relaxing effect (E_{MAX}) in gram-force (gf).

3.2. Vasoditator effect of (E,E)-farnesol on electro- and pharmacomechanical couplings of human umbilical arteries

In the presence of extracellular Ca^{2+}, (*E*,*E*)-FAR (1 μmol/L to 1 mmol/L) promoted total relaxation (E_{MAX} 100%) in arterial preparations subjected to electromechanical coupling by potassium chloride (KCl 60 mmol/L), in a concentration-dependent manner starting at 10 μmol/L ($p < 0.001$) and with EC_{50} 225.3 μmol/L (Fig. 2A). When evaluated alone, in the presence of calcium, the concentrations 800 μmol/L and 1 mmol/L had similar effects to that of nifedipine (10 μmol/L), and in the absence of Ca^{2+}, none of these agents promoted HUA ring relaxation (Fig. S1A).

In HUA rings stimulated with serotonin (5-HT 10 μmol/L), (*E*,*E*)-FAR (1 μmol/L to 1 mmol/L) also promoted concentration-dependent total relaxation (E_{MAX} 99.44%) starting at 10 μmol/L ($p < 0.001$), but with lower potency (EC_{50} 363.5 μmol/L) (Fig. 2B). In the absence of extracellular Ca^{2+}, preliminary experiments showed that the most effective concentrations of (*E*,*E*)-FAR (800 μmol/L and 1 mmol/L) when incubated separately promoted full relaxation of the preparations (Fig. S1B). Under these experimental conditions, the relaxing effect of (*E*,*E*)-FAR (1 mmol/L) did not differ from the effect of Y-27632 (10 μmol/L), a ROCK inhibitor, which also completely abolished contractile activity induced by 5-HT (Fig. 3).

Electromechanical and pharmacomechanical couplings were induced in HUA rings respectively by KCl (60 mmol/L) and 5-HT (10 μmol/L) in two different situations, in the presence (Krebs-Henseleit solution) and in the absence (Krebs-Henseleit solution without Ca^{2+}) of extracellular Ca^{2+}. Among these agents, 5-HT elicited the greatest contractile effect ($p < 0.05$). However consecutive applications reduced this response in the presence of Ca^{2+} compared to the response to KCl which remained constant. Both couplings were decreased in the absence of extracellular Ca^{2+}, 62.70% reduction for KCl and 43.83% for 5-HT (Fig. 4).

3.3. Involvement of ion channels (Ca^{2+} and K^{+}) in the vasodilator effect of (E,E)-farnesol in human umbilical arteries

After attesting to the vasodilator potential of (*E*,*E*)-FAR in the electro- and pharmacomechanical couplings. The two most effective concentrations in the concentration-response curve experiments (800 μmol/L and 1 mmol/L) were selected to investigate whether (*E*,*E*)-FAR at these concentrations interferes with Ca^{2+} homeostasis for the promotion of HUA ring relaxation, from modulation of ion channels (for Ca^{2+} or K^{+}) directly or indirectly. The cytosolic Ca^{2+} concentration is a determining factor for the contractile activity of HUA, while its increase leads to contraction, its decrease leads to relaxation.

In Ca^{2+}-free Krebs-Henseleit solution, HUA rings were depolarized by potassium (KCl 60 mmol/L), and after pre-incubation with (*E*,*E*)-FAR (800 μmol/L or 1 mmol/L) or nifedipine (10 μmol/L) for 25 ± 5 min, the preparations were cumulatively contracted with calcium chloride ($CaCl_2$ 0.1–20 mmol/L) to assess possible non-selective inhibition of Ca^{2+} channels (Fig. 5A), and in another experimental section, with barium chloride ($BaCl_2$ 0.1–20 mmol/L), to evaluate a possible inhibition of L-type voltage-operated Ca^{2+} channels (L-type VOCC) (Fig. 5B).

The Ca^{2+} ions for being non-selective exerted a greater contractile activity in the rings (E_{MAX} 2.16 gf) in a concentration-dependent manner (Fig. 5A), while the Ba^{2+} ions also presented this behavior, but for being selective for L-type VOCC this cation contracted the rings in only 1.84 gf of E_{MAX} (Fig. 5B), evidencing the contribution of this class of channels in the total contraction of the umbilical arteries. In the presence of Ca^{2+}, no concentration-dependent contractile changes were observed by any of these ions (Fig. S2).

(*E*,*E*)-FAR showed a relaxant effect ($p < 0.05$) in arterial preparations similar to the effect of nifedipine (10 μmol/L) in both Ca^{2+} and Ba^{2+} ion-induced contractions (Fig. 5A and B). (*E*,*E*)-FAR (800 μmol/L) reduced contractions by Ca^{2+} and Ba^{2+} ions more effectively than nifedipine with the lowest E_{MAX} values, confirming a possible inhibition of L-type VOCC and suggesting an inhibitory activity on other classes of Ca^{2+} channels, not evaluated in this study.

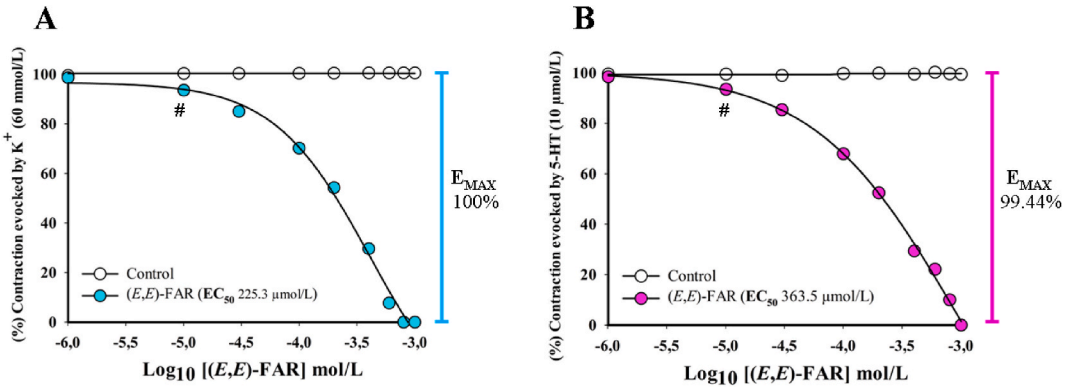

Fig. 2. Vasodilator effect of (*E*,*E*)-FAR on electromechanical and pharmacomechanical couplings of human umbilical arteries in the presence of Ca^{2+}. (**A**) Concentration-response curve of (*E*,*E*)-FAR (1 μmol/L to 1 mmol/L) on electromechanical coupling induced by KCl (60 mmol/L). (**B**) Concentration-response curve of (*E*,*E*)-FAR (1 μmol/L to 1 mmol/L) on pharmacomechanical coupling induced by 5-HT (10 μmol/L). Values expressed as mean ± S.E.M. ($n = 5$). The # symbol indicates that from this concentration the relaxation was significant ($p < 0.05$ *versus* control in two-way ANOVA followed by Holm-Sidak). The value of the median effective concentration (EC_{50}) is expressed in μmol/L and the maximum relaxing effect (E_{MAX}) in %.

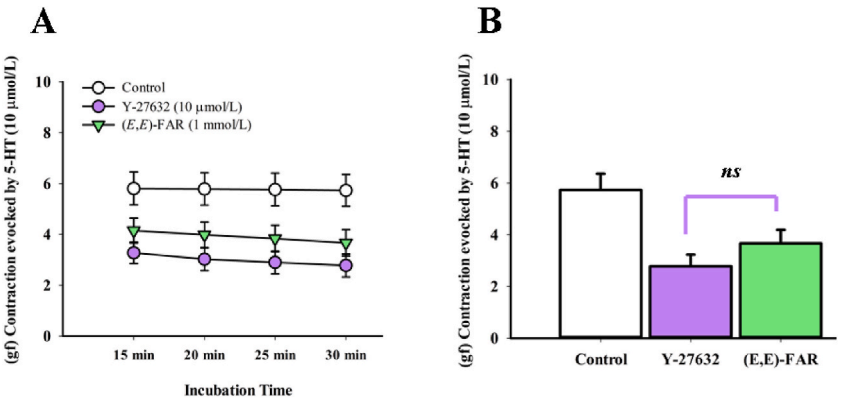

Fig. 3. Comparison of the vasodilator effect of (*E,E*)-FAR and the ROCK inhibitor (Y-27632) on the pharmacomechanical coupling of human umbilical arteries in the absence of Ca^{2+}. (**A**) Time-response curve of (*E,E*)-FAR (1 mmol/L) and Y-27632 (10 µmol/L) in the pharmacomechanical coupling induced by 5-HT (10 µmol/L). (**B**) Comparison of the vasodilator effect of (*E,E*)-FAR (1 mmol/L) and Y-27632 (10 µmol/L) on pharmacomechanical coupling induced by 5-HT (10 µmol/L) (incubation time: 30 min). Values expressed as mean ± S.E.M. ($n = 3$ replicates). The letters *ns* indicate absence of statistical difference ($p > 0.05$ *versus* Y-27632 in Student's *t*-test).

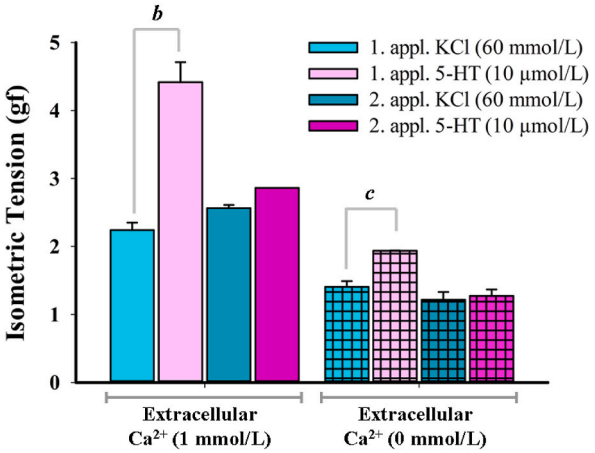

Fig. 4. Contractile effect of stimulation of human umbilical artery rings by KCl 60 mmol/L (electromechanical coupling) or 5-HT 10 µmol/L (pharmacomechanical coupling), in the presence and absence of Ca^{2+}. Two applications were conducted with each agent interleaved by Krebs-Henseleit solution washout and evaluated for 15 min each. Values expressed as mean ± S.E.M. ($n = 5$). Letters *b* and *c* indicate statistical significance ($p < 0.01$ and $p < 0.05$ KCl *versus* 5-HT in Student's t-test or Mann-Whitney *U* test).

Activation of K^+ channels also plays a critical role in HUA vasorelaxation, we investigated the possible involvement of different types of these channels by pre-incubating HUA rings with specific blockers predicted in the literature from human umbilical vessels for 25 ± 5 min, and subsequently contracting the preparations with 5-HT (10 µmol/L), and obtaining concentration-response curves with (*E,E*)-FAR (1 µmol/L to 1 mmol/L). The potency and efficacy measures of (*E,E*)-FAR in the presence of blockers are summarized in Table 1.

The blockers used, tetraethylammonium - TEA (1 mmol/L for large conductance Ca^{2+}-activated K^+ channels - BK_{Ca} and voltage-dependent K^+ channels - K_V, or 10 mmol/L for non-selective blockade), 4-aminopyridine - 4-AP (1 mmol/L for K_V channels) and glibenclamide - GLI (10 µmol/L for ATP-dependent K^+ channels - K_{ATP}) did not affect basal tone, except $BaCl_2$ (500 µmol/L for inward rectifier K^+ channels - K_{IR}) which promoted contractile activity ($p < 0.008$) in basal tone (Fig. 6A). None of the blockers affected the response to 5-HT (Fig. 6B).

For all arterial preparations submitted to each one of the K^+ channel blockers, there was an alteration in the pharmacodynamic parameters of relaxing potency (EC_{50}) and efficacy (E_{MAX}) of (*E,E*)-FAR (1 µmol/L to 1 mmol/L) but its concentration-dependent behavior was not affected (Fig. 7). An increase in the EC_{50} values of (*E,E*)-FAR was observed in the presence of all blockers, indicating a reduction in relaxing potency and possible participation of different K^+ channels (BK_{Ca}, K_V, K_{ATP}, and K_{IR}) in this effect.

In parallel, the E_{MAX} values in these preparations decreased indicating also a reduction in relaxant efficacy, except for the rings pre-incubated with $BaCl_2$ (500 µmol/L) in which this agent did not infer on the relaxant efficacy promoted by (*E,E*)-FAR (E_{MAX} 99.44%) (Fig. 7). Interestingly, in HUA rings pre-incubated with 4-AP (1 mmol/L) or $BaCl_2$ (500 µmol/L), (*E,E*)-FAR promoted relaxation from

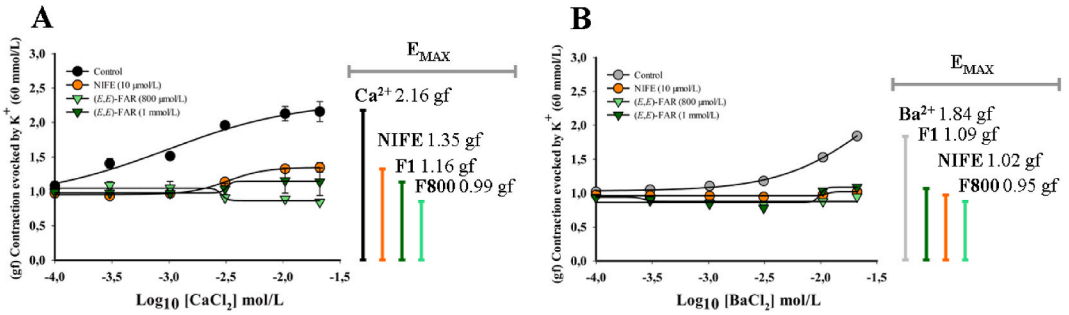

Fig. 5. Involvement of Ca^{2+} channels in the vasodilator effect of (*E*,*E*)-FAR (800 μmol/L or 1 mmol/L) on contractions induced by $CaCl_2$ or $BaCl_2$ (0.1–20 mmol/L) in human umbilical arteries. (**A**) Concentration-response curves promoted by $CaCl_2$ in the presence of (*E*,*E*)-FAR (800 μmol/L or 1 mmol/L) or nifedipine (10 μmol/L), in an extracellular Ca^{2+}-free environment. (**B**) Concentration-response curves promoted by $BaCl_2$ in the presence of (*E*,*E*)-FAR (800 μmol/L or 1 mmol/L) or nifedipine (10 μmol/L), in an extracellular Ca^{2+}-free environment. Values expressed as mean ± S.E.M. ($n = 5$). The value of the maximum contractile effect (E_{MAX}) is expressed in gf. F800 is abbreviation for (*E*,*E*)-FAR 800 μmol/L, F1 for (*E*,*E*)-FAR 1 mmol/L, and NIFE for nifedipine.

Table 1
Pharmacodynamic parameters of (*E*,*E*)-FAR (1 μmol/L to 1 mmol/L) in the presence of different human umbilical artery contractile agents.

Contractile Agents	Pharmacodynamic Parameters of (*E*,*E*)-FAR[1]		
	EC_{50}	E_{MAX}	[(*E*,*E*)-FAR] $p < 0,05$
KCl (60 mmol/L)	225.3 μmol/L	100%	10 μmol/L
5- HT (10 μmol/L)	363.5 μmol/L	99.44%	10 μmol/L
TEA (10 mmol/L)	622.2 μmol/L	77.47%	100 μmol/L
TEA (1 mmol/L)	397.8 μmol/L	82.16%	10 μmol/L
4-AP (1 mmol/L)	460.9 μmol/L	74.11%	1 μmol/L
GLI (10 μmol/L)	697.1 μmol/L	77.62%	10 μmol/L
$BaCl_2$ (500 μmol/L)	984.1 μmol/L	99.44%	1 μmol/L

[1] The table shows a summary of the pharmacodynamic parameters of (*E*,*E*)-farnesol (1 μmol/L to 1 mmol/L) investigated for each of the contractile agents studied in the concentration-response curves: The median effective concentration (EC_{50}) indicates pharmacological potency, the maximum effect (E_{MAX}) indicates pharmacological efficacy, and statistically significant concentrations compared to control ([(*E*,*E*)-FAR] $p < 0.05$) indicate from which point of the (*E*,*E*)-FAR curve relaxation starts. KCl (potassium chloride); 5-HT (5-hydroxytryptamine/serotonin); TEA (tetraethylammonium); 4-AP (4-aminopyridine); GLI (glibenclamide); $BaCl_2$ (barium chloride).

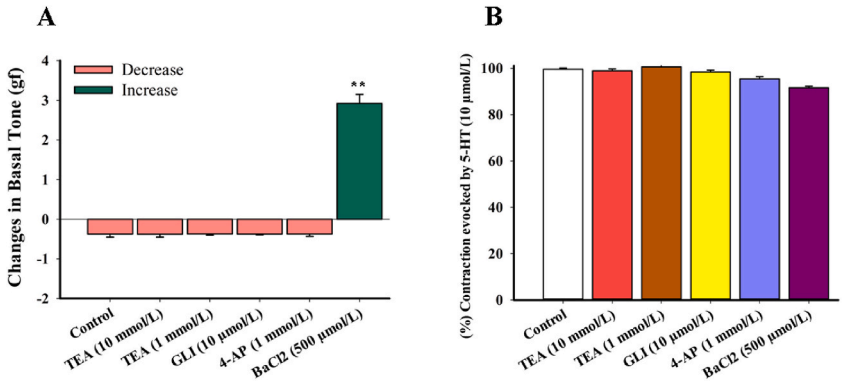

Fig. 6. Effect of K^+ channel blockers on basal tone and contraction by 5-HT (10 μmol/L) of human umbilical arteries. (**A**) Changes in basal tone produced by K^+ channel blockers with trends toward decreased or increased contractile tension (in gf). (**B**) Effect of K^+ channel blockers on contraction induced by 5-HT (10 μmol/L) presented in %. Values expressed as mean ± S.E.M. ($n = 7$). The ** symbol indicates statistical significance ($p < 0.01$ *versus* control in Student's *t*-test or Mann-Whitney *U* test).

the concentration of 1 μmol/L ($p < 0.05$) (Table 1).

4. Discussion

Human umbilical arteries (HUA) are present in pairs in the umbilical cord, and are responsible for transporting oxygen-poor blood

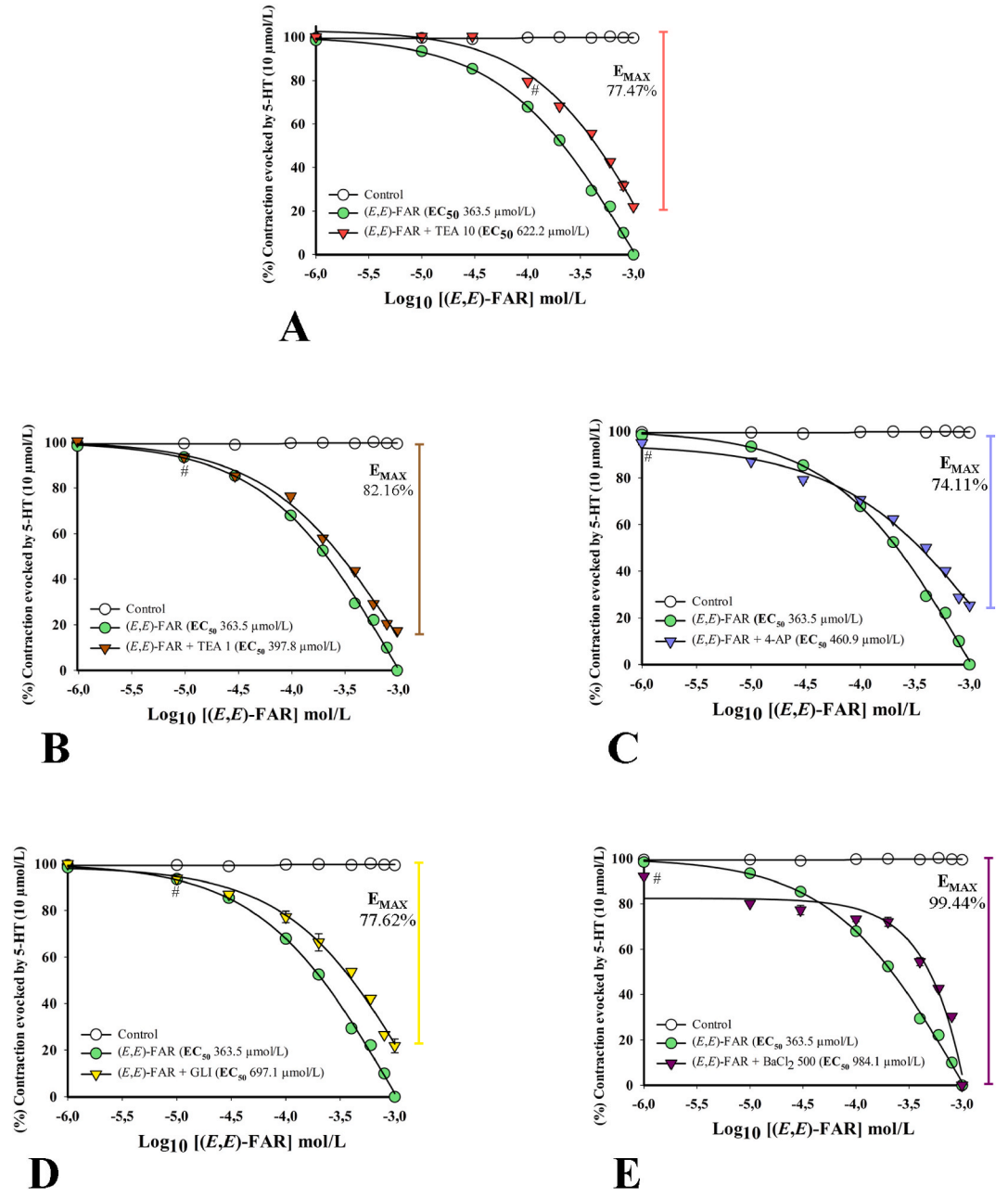

Fig. 7. Involvement of K^+ channels in the vasodilator effect of (*E*,*E*)-FAR (1 µmol/L to 1 mmol/L) in the presence of different K^+ channel blockers (incubation period: 25 ± 5 min) in human umbilical arteries. (**A**) Effect of (*E*,*E*)-FAR on 5-HT (10 µmol/L) induced contractions in human umbilical artery rings pre-incubated with TEA (10 mmol/L). (**B**) Effect of (*E*,*E*)-FAR on 5-HT (10 µmol/L) induced contractions in human umbilical artery rings pre-incubated with TEA (1 mmol/L). (**C**) Effect of (*E*,*E*)-FAR on 5-HT-induced contractions (10 µmol/L) in human umbilical artery rings pre-incubated with 4-AP (1 mmol/L). (**D**) Effect of (*E*,*E*)-FAR on 5-HT (10 µmol/L) induced contractions in human umbilical artery rings pre-incubated with GLI (10 µmol/L). (**E**) Effect of (*E*,*E*)-FAR on 5-HT (10 µmol/L) induced contractions in human umbilical artery rings pre-incubated with $BaCl_2$ (500 µmol/L). Values expressed as mean ± S.E.M. ($n = 5$). The # symbol indicates that from this concentration the relaxation was significant ($p < 0.05$ *versus* control in two-way ANOVA followed by Holm-Sidak). The value of the median effective concentration (EC_{50}) is expressed in µmol/L and the maximum relaxing effect (E_{MAX}) in %.

from the fetus to the placenta. Anatomically, it is a medium muscular artery (1–10 mm) formed by three tunics (intima, media and adventitia), among which the media layer is responsible for contractile activity [21]. Besides their physiological role, they are easy to obtain and applicable to cardiovascular studies [22].

Because they do not have nerve endings, the regulation of their contractility is dependent on ionic flows (Ca^{2+} and K^+) and local

vasoactive mediators or existing in the fetoplacental circulation, such as serotonin (5-HT), histamine, thromboxane, bradykinin, endothelin 1 and prostaglandin F2α that are linked to the activation of G_q and $G_{i/o}$ proteins [23]. In this context, studies that elucidate the interference of natural products in the regulatory mechanisms of HUA contractility are promising to substantiate the therapeutic action of these products in the treatment of disorders that increase the resistance of these vessels, such as gestational hypertension and preeclampsia.

This is the first publication describing the vasodilatory effect of (*E,E*)-FAR on HUA from healthy women. Our main findings show that (*E,E*)-FAR promotes reversible relaxation in basal tone of HUA, and is able to reduce all contractile activity induced by depolarization (KCl 60 mmol/L) or pharmacological agonism (5-HT 10 μmol/L), with higher potency in the electromechanical pathway, even compared with the relaxing pharmacological potencies of other natural products in HUA [16,17].

Cumulative addition of (*E,E*)-FAR from 1 μmol/L to 1 mmol/L reduced basal tone of HUA in a concentration-dependent and partially reversible manner (above the tissue viability threshold). In previous studies, (*E,E*)-FAR was able to increase the resting internal diameter of human (resistance) arteries [9] and reduce the intravascular free and basal [Ca^{2+}] of rat mesenteric arteries [10]. Our results also corroborate with other reports showing that the basal tone of HUA is altered by natural products, such as (−)-carveol (monoterpene) [17] and eugenol (phenylpropanoid) [16].

In general, under *in vivo* conditions, the vascular tonus is the sum of three components: (1) the basal or resting tonus (myogenic and intrinsic) dependent on stretch (transmural pressure) and flow (shear forces); (2) the tonus induced by agonists; (3) and the matrix elements in the vessel wall [24–26]. In this sense, experimental evaluations on the basal tone may have some clinical utility, since in the physiopathology of hypertensive disorders in pregnancy, such as preeclampsia, there is a decrease in the activity of the Na^+/Ca^{2+} exchanger (NCX) in HUA that may be implicated in an abnormal regulation of the basal tone [27,28].

In the electromechanical pathway, (*E,E*)-FAR (1 μmol/L to 1 mmol/L) promoted concentration-dependent relaxation only in the presence of extracellular Ca^{2+}, as did nifedipine (10 μmol/L), an L-type VOCC blocker used as a control based on previous studies [29,30]. These results indicate that the main mechanism of (*E,E*)-FAR for promoting relaxation of electromechanical contractions in HUA resembles that of nifedipine, i.e., inhibiting L-type VOCC and thereby reducing extracellular Ca^{2+} influx, and consequently, $[Ca^{2+}]_i$.

This mechanism in common could also explain why the vasodilator effect of (*E,E*)-FAR was partially reversible in our results, because in a previous study, it was noted that the inhibitory effect of nifedipine of K^+-induced contractions in HUA, was also not fully reversed even after washing the preparations for up to 90 min [31].

Indeed, the literature describes that (*E,E*)-FAR is able to inhibit KCl-induced contractions in rat aortic rings in a time- and dose-dependent manner [9], moreover, in A7r5 cells, derived from rat aortic vascular smooth muscle, it inhibited (~80%) the KCl-induced increase in $[Ca^{2+}]_i$ [11].

In the pharmacomechanical pathway, (*E,E*)-FAR (1 μmol/L to 1 mmol/L) although less potent, also promoted concentration-dependent relaxation in the presence and absence of Ca^{2+}. These data reinforce what has already been predicted for the vasorelaxant action of (*E,E*)-FAR in rat aortic rings and human resistance arteries, showing that in addition to the direct modulation of Ca^{2+} channels, it acts to some degree in G protein-dependent pathways and Ca^{2+} release from intracellular stores to inhibit pharmacomechanical contractions [9]. Although no vascular physiopharmacology studies exploring the interaction of (*E,E*)-FAR with serotoninergic receptors are found, we do not rule out this hypothesis as there is evidence of its interaction with at least the 5-HT_3 isoform [32].

In the absence of extracellular Ca^{2+}, (*E,E*)-FAR produced relaxation only in pharmacomechanical coupling (supplementary materials), which may suggest that its inhibitory effect on 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase that converts HMG-CoA to mevalonate, could reduce the levels of farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GPP) that participate in the prenylation of various proteins [33], and consequently affect the activation of the ROCK pathway implicated in HUA contraction in the absence of Ca^{2+} [34,35]. The presence of HMG-CoA reductase in HUA smooth muscle cells has already been considered by another study [36]. However, more studies are needed to confirm this hypothesis.

To analyze the vasodilatory effect of (*E,E*)-FAR on electromechanical and pharmacomechanical excitations-contractions, we pre-contracted HUA rings with KCl (60 mmol/L) or 5-HT (10 μmol/L) prior to its addition, in the presence and absence of extracellular Ca^{2+}. The increase in $[Ca^{2+}]_i$ is critical for the initiation of contractile activity, in this context, the use of KCl to evoke contractions in HUA is well documented in the literature, whose suggested mechanism is due to the influx of extracellular Ca^{2+} through VOCC and inactivation of K_V channels [23,30].

In turn, 5-HT is considered the most potent vasoconstrictor in HUA and is responsible for the physiological closure of HUA after delivery. This biogenic monoamine triggers the contractile process by activation of 5-HT_{2A} receptors that drive the $G_q/PLC/IP_3$ pathway and $5\text{-HT}_{1B/1D}$ that drive the $G_{i/o}/\downarrow AC/\downarrow AMPc$ pathway, and consequently, increases $[Ca^{2+}]_i$. Secondarily, activation of Ca^{2+} channels, inhibition of K_V channels, and endogenous production of thromboxane A_2 may also be associated with the process [23,29,30,37].

In our experimental conditions, the tension of contractions from 5-HT stimulation were higher than that from KCl, a pattern already observed by our group [16,17] and consistent with other studies [38,39]. However, after repeated KCl incubations, the contractile response remained constant compared to the 5-HT response, which had a decreasing trend. Similar data were found by Tufan et al. [29].

In the absence of Ca^{2+}, there was a contractile response to both agents (KCl and 5-HT), but in a diminished form from the first application compared to when the extracellular medium has Ca^{2+} available. In this case, the maintenance of contractile activity in response to KCl may be due to the participation of Ca^{2+}-independent isozymes of protein kinase C, the progressive release of Ca^{2+} from intracellular stores and activation of RhoA-kinase (ROCK) with consequent inhibition of myosin light chain phosphatase (Ca^{2+} sensitization), these last two mechanisms are also implicated in contractions (of phasic and tonic components) induced by 5-HT in the

absence of Ca^{2+} [29,30].

The greater pharmacological potency of (*E*,*E*)-FAR in the electromechanical pathway in HUA directed us to investigate the influence of Ca^{2+} and K^+ ion channels on the vasodilator effect. We observed that (*E*,*E*)-FAR (800 μmol/L) suppressed contractions evoked by Ca^{2+} and Ba^{2+} ions more effectively than the control nifedipine which, in turn, is a potent inhibitor of Ca^{2+} [31] and Ba^{2+} [16,17,19] induced contractions in HUA.

These data corroborate the findings Luft et al. [12] who confirmed the inhibitory action of farnesol (isomer not described) on (*C*-class) L-type Ca^{2+} channels with pharmacodynamics partly similar to that of dihydropyridine antagonists. On the other hand, our data also support the hypothesis that (*E*,*E*)-FAR may block other types of voltage-dependent Ca^{2+} channels expressed in HUA, such as P-/Q-types of the high voltage-activated family (HVA) or T-type of the low voltage-activated family (LVA) [40].

This hypothesis is supported by the fact that in neuronal ion channels, micromolar concentrations of (*E*,*E*)-FAR blocked all types of HVA channels, and at nanomolar concentrations, it selectively inhibited N-type voltage-dependent Ca^{2+} channels [41]. Furthermore, in human retinal glial cells, (*E*,*E*)-FAR promoted a reduction of currents mediated by HVA and LVA Ca^{2+} channels [42].

Our latest experiments evaluated the involvement of a variety of K^+ channels (BK_{Ca}, K_V, K_{ATP} and K_{IR}) in the vasodilator mechanism of (*E*,*E*)-FAR. None of the blockers used (TEA, 4-AP or GLI) affected basal tone or pharmacomechanical coupling produced by 5-HT, except blockade by $BaCl_2$ (500 μmol/L) which evoked contraction in basal tone, all of these data are similar to other investigations [20,39,43].

The activity of K^+ channels leads to hyperpolarization, VOCC closure, and vasorelaxation, and is one of the main mechanisms regulating resting membrane potential (MP) and vascular tone under (patho-) physiological conditions, and for this reason are promising therapeutic targets in clinical practice, including for vascular diseases in pregnancy in which their expression and function are altered. Among the types of these channels in HUA, there are K_V, BK_{Ca}, K_{IR} and K_{ATP}. K_V channels open in response to depolarization, BK_{Ca} in response to increased $[Ca^{2+}]_i$, K_{IR} in response to the difference between the MP and the K^+ equilibrium potential (E_K), and K_{ATP} in response to the ATP/ADP ratio [44].

All blockers tested affected the efficacy and potency of the relaxant response of (*E*,*E*)-FAR suggesting a possible activation of these channels, a mechanism similar to that of other natural products in HUA [16,17]. In other cellular systems, activation of K_{ATP} by farnesol is hypothesized [32], corroborating with our results. If indeed (*E*,*E*)-FAR is able to promote the opening of K_{ATP} channels, this would also explain our results on the reduction promoted by (*E*,*E*)-FAR in 5-HT-induced pharmacomechanical coupling in the absence of extracellular Ca^{2+}, since K_{ATP} channel openers (such as levcromakalim) abolished 5-HT-induced contractions in Ca^{2+}-free solution, possibly by inhibiting Ca^{2+} release and refilling from intracellular stores critical for the contractile process under these experimental conditions [29].

The literature also reports the activation of BK_{Ca} channels by terpene derivatives, flavonoids and naturally occurring phenolic derivatives [17,44]. As for the action of (*E*,*E*)-FAR in the presence of K_V and K_{IR} channel blockers, it remains uncertain and deserves future molecular-based investigations, since in this case, relaxation was evident from 1 μmol/L of (*E*,*E*)-FAR.

Possibly a complex modulation of the internal rectification of K_{IR} channels by (*E*,*E*)-FAR may be somehow associated with our results, the suggested mechanisms of which are beyond the scope of the present study. Another hypothesis would be that the increase in K_V channel activity promoted by (*E*,*E*)-FAR was potentiated by 4-AP, there are precedents [45].

The physiopharmacological implications of the data presented in the present study come down to the fact that most research aimed at the discovery of vasorelaxant biomolecules employs non-human animal tissues. However, the mechanisms involved in the relaxant response are different in human tissues. Thus, the use of human umbilical vessels is relevant in this sense, because the vascular reactivity of fetal and maternal circulations are not identical, and umbilical vessels respond poorly to some relaxing compounds that are effective in other systemic vessels [16]. In this context, characterization of the vasodilatory effect of (*E*,*E*)-FAR in HUA highlights its importance amidst the gaps in the literature of vasoactive natural products in HUA.

5. Conclusions

In conclusion, our data show that (*E*,*E*)-FAR has a vasodilator effect on HUA smooth muscle and affects basal tone, being most effective and potent in the electromechanical excitation-contraction pathway. Direct inhibition of L-type VOCC seems to be the main mechanism of this effect, but inhibition of other types of voltage-dependent channels (P-/Q and T) cannot be ruled out. In Ca^{2+}-free medium, (*E*,*E*)-FAR probably interferes with the ROCK pathway of pharmacomechanical contraction. Furthermore, activation of different K+ channels (BK_{Ca}, K_V, K_{ATP} and K_{IR}) seems to contribute to the relaxation of (*E*,*E*)-FAR. These results suggest that (*E*,*E*)-FAR may be a promising pharmacological candidate to be applicable in obstetric hypertensive disorders that increase umbilical artery resistance.

Author contribution statement

Paulo Ricardo Batista: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Andressa de Alencar Silva; Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Carla Mikevely de Sena Bastos; Gabriela Lucena Calixto: Performed the experiments.

Marta Regina Kerntopf; Renata Evaristo Rodrigues da Silva; Luís Pereira de Morais; Gyllyandeson de Araújo Delmondes: Contributed reagents, materials, analysis tools or data.

Roseli Barbosa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Irwin Rose Alencar de Menezes: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17328.

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