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Original Article

Evaluation of activity inotropic of a new steroid derivative using an isolated rat heart model

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Abstract: There are studies which indicate that some steroid derivatives have inotropic activity; nevertheless, the cellular site and mechanism of action at cardiovascular level is very confusing. In order, to clarify these phenomena in this study, a new estradiol derivative was synthesized with the objective of to evaluate its biological activity on left ventricular pressure and characterize their molecular mechanism. The Langendorff technique was used to measure changes on perfusion pressure and coronary resistance in an isolated rat heart model in absence or presence of the estradiol derivative. Additionally, to characterize the molecular mechanism involved in the inotropic activity induced by the OTBDS-estradiol-hexanoic acid derivative was evaluated by measuring left ventricular pressure in absence or presence of following compounds; tamoxifen, prazosin, metoprolol, indomethacin and nifedipine. The results showed that the OTBDS-estradiol-hexanoic acid derivative significantly increased the perfusion pressure and coronary resistance in comparison with the control conditions. Additionally, other data indicate that OTBDS-estradiol-hexanoic acid derivative increase left ventricular pressure in a dose-dependent manner (0.001 to 100 nM); nevertheless, this phenomenon was significantly inhibited only by nifedipine at a dose of 1 nM. These data suggest that positive inotropic activity induced by the OTBDS-estradiol-hexanoic acid derivative is via activation of L-type calcium channel. This phenomenon is a particularly interesting because the positive inotropic activity induced by this steroid derivative involves a molecular mechanism different in comparison with other positive inotropic drugs.

Keywords: Steroid derivative, inotropic activity, indomethacin, nifedipine

Introduction

Since several years ago, diverse drugs have been developed to treatment of congestive heart failure (CHF); for example, the synthesis of compound (-)-(R)-1-(p-hydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino]-ethanol (TA-064) which exerts a positive inotropic activity in isolated guinea pig heart [1]. Other studies indicate that the compounds MDL 17,043 [1,3-dihydro-4-methyl-5-[4-(methylthio)-benzoyl]-2H-imidazol-2-one] and AR-L 115 BS [sulmazole, 2-[(2-methoxy-4-methylsulfinyl)phenyl]-1H-imidazo[4,5-b] pyridine] exerts positive inotropic effects in an isolated canine ventricular trabeculae model [2]. In addition, data indicate that a dihydropyridine derivative (Bay k 8644) induce positive inotropic activity in cells myocardial through of calcium channels activation [3].

On the other hand, the positive inotropic activity of a series of steroid derivatives has been evaluated in diverse biological models; for example, a study indicates that 14β-hydroxyprogesterone [4] increases the contractility of isolated cardiac tissue via glycoside receptor. Other studies showed that 20R 14β-amino-3β-rhamnosyl-5β-pregnan-20β-ol can also induce a positive inotropic action in a dog model of induced heart

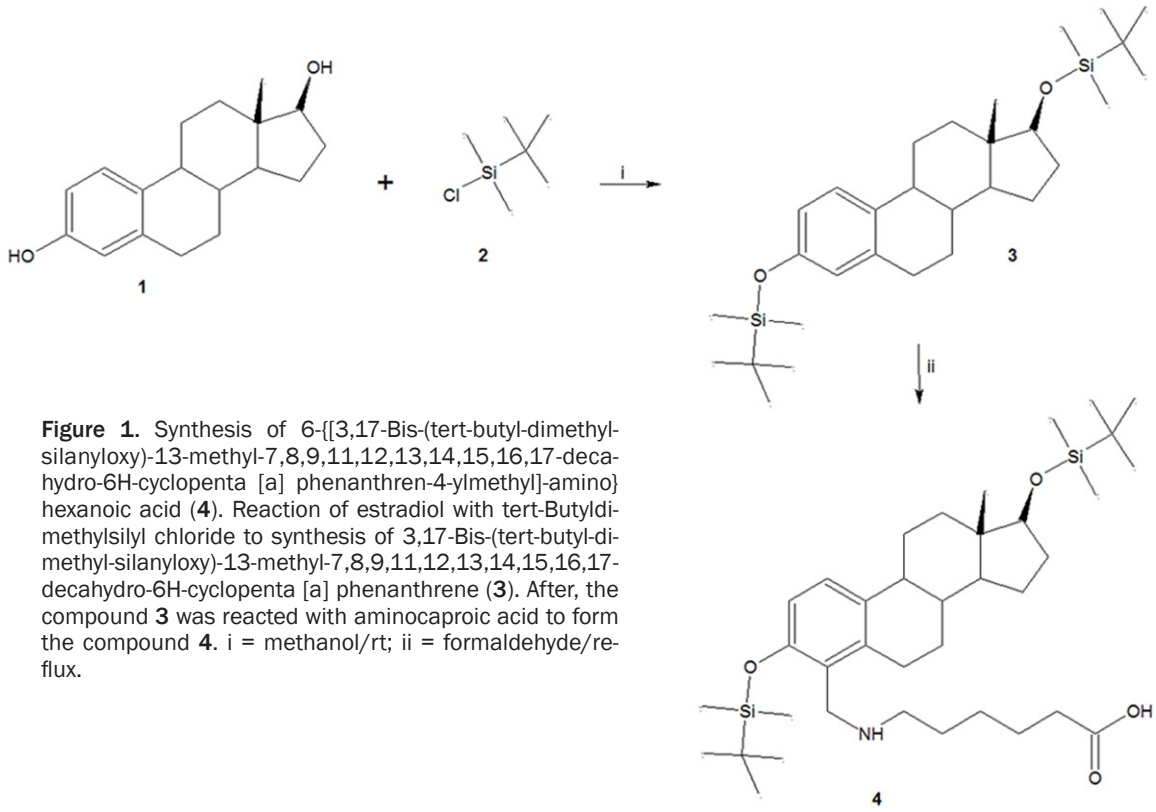

Figure 1. Synthesis of 6-{[3,17-Bis-(tert-butyl-dimethyl-silanyloxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-deca-hydro-6H-cyclopenta [a] phenanthren-4-ylmethyl]-amino} hexanoic acid (**4**). Reaction of estradiol with tert-Butyldi-methylsilyl chloride to synthesis of 3,17-Bis-(tert-butyl-di-methyl-silanyloxy)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta [a] phenanthrene (**3**). After, the compound **3** was reacted with aminocaproic acid to form the compound **4**. i = methanol/rt; ii = formaldehyde/re-flux.

failure and this effect could be through its high affinity to Na^+, K^+-ATPase receptor [5]. Additionally, there data which indicate that other type of steroid derivative (strophanthidin) increase the force of contraction by changes in the calcium levels [6]. Other studies show the preparation of F90927 (a steroid derivative) which exerts a positive inotropic activity in cardiac muscle via activation of the L-type Ca^{2+} channel [7]. All these data show that some steroid derivatives induce inotropic effects in the cardiovascular system; nevertheless, the cellular site and molecular mechanism involved in its inotropic activity are very confusing, perhaps this phenomenon is due to differences in the chemical structure of steroid derivatives or to the different pharmacological approaches used. Therefore, more pharmacological data are needed to characterize the activity induced by the steroid derivatives at cardiovascular level. To provide this information, the present study was designed to investigate the effects of an estradiol derivative on perfusion pressure and coronary resistance in isolated rat hearts using the Langendorff technique. In addition, to evaluate the molecular mechanism involved in the inotropic activity induced by the estradiol derivative on left ventricular pressure the following compounds were used as pharmacological tools; tamoxifen (antagonist of estrogen-receptors) [8], prazosin (α_1 adrenoreceptor antagonist) [9], metoprolol (selective β_1 receptor blocker) [10], indomethacin (a nonselective inhibitor of cyclooxygenase) [11] and nifedipine (antagonist of calcium-channel) [12].

Material and methods

Chemical synthesis

The compounds evaluated in this study were purchased from Sigma-Aldrich Co., Ltd. The melting point for the triazole derivative was determined on an Electrothermal (900 model). ^1H and ^{13}C NMR (nuclear magnetic resonance) spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz (megahertz) in $CDCl_3$ (deuterated chloroform) using TMS (tetramethylsilane) as internal standard. EIMS (electron impact mass spectroscopy) spectra were obtained with a Finnigan Trace Gas Chromatography Polaris Q Spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/0 2400 elemental analyzer.

Table 1. ^{1}H NMR (300 MHz, $CDCl_3$) data for the Estradiol-OTBDS derivative

0.06 (s, 6H), 0.26 (s, 6H), 0.84 (s, 3H), 0.88 (s, 9H), 1.02 (m, 1H), 1.06 (s, 9H), 1.10-1.66 (m, 7H), 1.88-2.00 (m, 4H), 2.10-2.80 (m, 3H), 3.50 (m, 1H), 6.80-7.20 (m, 3H) ppm.

Table 2. ^{1}H NMR (75.4 MHz, $CDCl_3$) data for the Estradiol-OTBDS derivative

-4.43, -4.39, 11.30, 18.00, 18.10, 23.60, 25.50, 25.60, 26.29, 27.30, 29.56, 31.05, 38.40, 39.00, 43.16, 44.26, 51.10, 81.60, 117.10, 119.90, 126.28, 132.50, 137.90, 153.28 ppm.

Table 3. ^{1}H NMR (300 MHz, $CDCl_3$) data for the OTBDS-estradiol-hexanoic acid derivative

0.06 (s, 6H), 0.26 (s, 6H), 0.84 (s, 3H), 0.88 (s, 9H), 0.98 (s, 9H), 1.02 (m, 1H), 1.20-1.22 (m, 2H), 1.26 (t, 2H J = 0.25 Hz), 1.34-1.38 (m, 2H), 1.48 (t, 2H J = 0.50 Hz), 1.56-1.60 (m, 2H), 1.64 (t, 2H J = 0.25 Hz), 1.70-2.10 (m, 5H), 2.40 (t, 2H J = 0.25 Hz), 2.44-2.54 (m, 3H), 2.72 (t, 2H J = 0.63 Hz), 3.50 (m, 1H), 3.64 (m, 2H), 6.10 (broad, 2H), 6.60-6.80 (m, 2H) ppm

Table 4. ^{1}H NMR (75.4 MHz, $CDCl_3$) data for the OTBDS-estradiol-hexanoic acid derivative

-4.39, -4.2, 11.30, 18.00, 18.410, 23.27, 25.22, 25.40, 25.50, 25.70, 26.10, 27.50, 27.69, 27.71, 31.05, 33.50, 38.40, 38.92, 43.10, 44.50, 45.90, 50.00, 51.10, 81.68, 114.70, 124.00, 127.22, 130.80, 136.40, 151.00, 175.28 ppm

Synthesis of 3, 17-Bis-(tert-butyl-dimethyl-silanyloxy)-13-methyl-7,8,9,11,12,13,14,15, 16,17-decahydro-6H-cyclopenta[a]phenan-threne (compound 3)

A solution of estradiol (100 mg, 0.37 mmol), tert-Butyldimethylsilyl chloride (200 µl, 1.15 mmol) in 5 ml of methanol was stirred for 24 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure, the residue washed 3 times with water. Then the precipitate was separated and dried at room temperature.

Synthesis of 6-{[3,17-Bis-(tert-butyl-dimethyl-silanyloxy)-13-methyl-7,8,9,11,12,13,14,15, 16,17-decahydro-6H-cyclopenta[a]phenan-thren-4-ylmethyl]-amino}hexanoic acid

A solution of compound 3 (100 mg, 0.20 mmol), 6-aminohexanoic acid (50 mg, 0.38 mmol) in 10 ml of formaldehyde was stirred for 24 h to reflux. The reaction mixture was evaporated to dryness under reduced pressure, the residue washed 3 times with water. Then the precipitate was separated and dried at room temperature.

Biological method

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal care and use Committee of University Autonomous of Campeche (No. PI-420/12) and were in accordance with the guide for the care and use of laboratory animals [13]. Male Wistar rats; weighing 200-250 g were obtained from University Autonomous of Campeche.

Reagents

All drugs were dissolved in methanol and different dilutions were obtained using Krebs-Henseleit solution (≤ 0.01%, v/v).

Experimental design

Briefly, the male rat (200-250 g) was anesthetized by injecting them with pentobarbital at a dose rate of 50 mg/Kg body weight. Then the chest was opened, and a loose ligature passed through the ascending aorta. The heart was then rapidly removed and immersed in ice cold physiologic saline solution. The heart was trimmed of non-cardiac tissue and retrograde perfused via a non-circulating perfusion system at a constant flow rate. The perfusion medium was the Krebs-Henseleit solution (pH = 7.4, 37°C) composed of (mmol); 117.8 NaCl; 6 KCl; 1.75 $CaCl_2$; 1.2 NaH_2PO_4; 1.2 $MgSO_4$; 24.2 $NaHCO_3$; 5 glucose and 5 sodium pyruvate. The solution was actively bubbled with a mixture of O_2/CO_2 (95:5/5 %). The coronary flow was adjusted with a variable speed peristaltic pump. An initial perfusion rate of 15 ml/min for

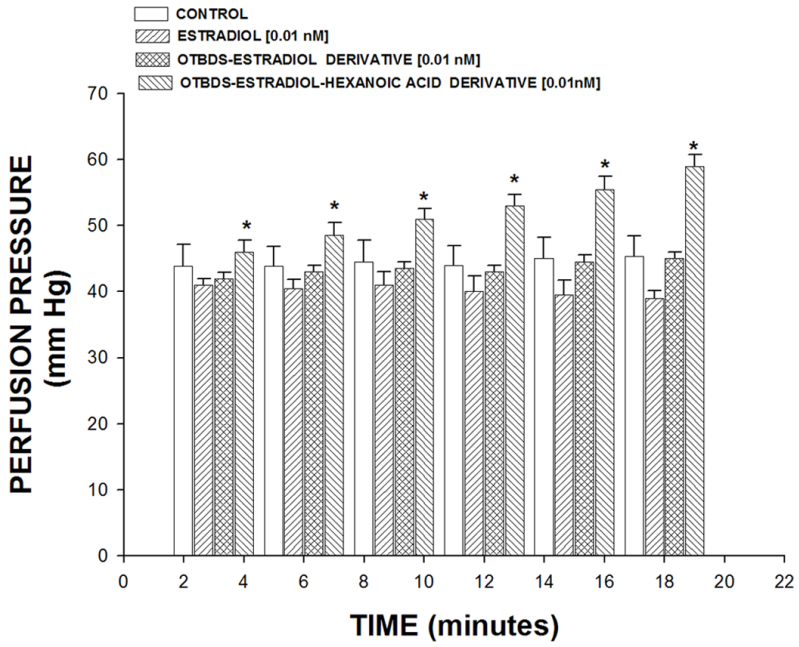

Figure 2. *Effect of estradiol and its derivatives on perfusion pressure.* The results show that the OTBDS-estradiol-hexanoic acid derivative significantly increase perfusion pressure ($P = 0.05$) through time in comparison with estradiol, OTBDS-estradiol derivative and the control conditions. Each bar represents the mean ± S.E. of 9 experiments.

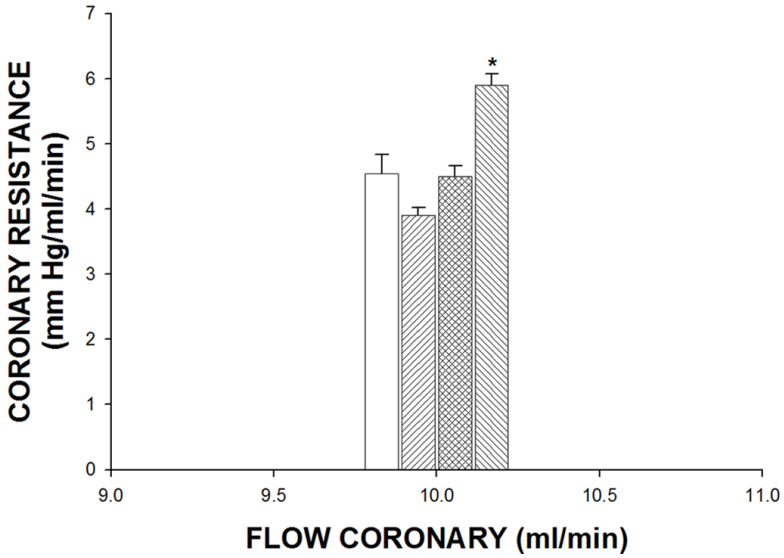

Figure 3. *Activity exerted by estradiol and its derivatives on coronary resistance.* The results show that coronary resistance was higher ($P = 0.06$) in the presence of the OTBDS-estradiol-hexanoic acid derivative in comparison with estradiol, OTBDS-estradiol derivative and the control conditions. Each bar represents the mean ± S.E. of 9 experiments.

5 min was followed by a 15 min equilibration period at a perfusion rate of 10 ml/min. All experimental measurements were done after this equilibration period.

Perfusion pressure

Evaluation of measurements of perfusion pressure changes induced by drugs administration in this study were assessed using a pressure transducer connected to the chamber where the hearts were mounted and the results entered into a computerized data capture system (Biopac).

Inotropic activity

Contractile function was assessed by measuring left ventricular developed pressure (LV/dP), using a saline-filled latex balloon (0.01 mm, diameter) inserted into the left ventricle via the left atrium. The latex balloon was bound to cannula which was linked to pressure transducer that was connected with the MP100 data acquisition system.

Biological evaluation

First stage

Effects of estradiol, estradiol-OTBDS derivative and OTBDS-estradiol-hexanoic acid *derivative on perfusion pressure*: Changes in perfusion pressure as a consequence of increases in time (3 to 18 min) in absence (control) and presence of estradiol and its derivatives at a concentration of 0.001 nM were determined. The effects were obtained in isolated hearts perfused at a constant-flow rate of 10 ml/min.

Evaluation of effects exerted by estradiol, estradiol-OTBDS derivative and OTBDS-estradiol-hexanoic acid *derivative on coronary resistance*: The coronary resistance in absence

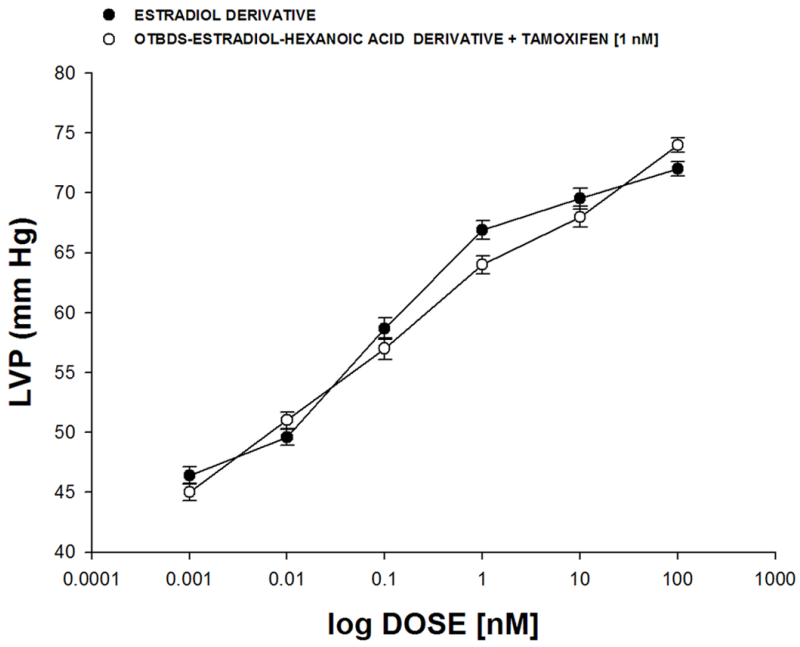

Figure 4. *Effect of OTBDS-estradiol-hexanoic acid derivative on LVP through estrogen receptors*. OTBDS-estradiol-hexanoic acid derivative [0.001 to 100 nM] was administered (intracoronary boluses, 50 µl) and the corresponding effect on the LVP was evaluated in the absence and presence of tamoxifen. The results showed that activity induced by the OTBDS-estradiol-hexanoic acid derivative on LVP was not inhibited in the presence of tamoxifen. Each bar represents the mean ± S.E. of 9 experiments. LVP = left ventricular pressure.

(control) and presence of estradiol and its derivatives at a concentration of 0.001 nM was evaluated. The effects were obtained in isolated hearts perfused at a constant flow rate of 10 ml/min. Since a constant flow was used changes in coronary pressure reflected the changes in coronary resistance.

<u>*Second stage*</u>

Effects of OTBDS-estradiol-hexanoic acid *derivative on left ventricular pressure through estrogen receptor activation*: Intracoronary boluses (50 µl) of the OTBDS-estradiol-hexanoic acid derivative (0.001 to 100 nM) were administered and the corresponding effect on the left ventricular pressure was determined. The dose-response curve (control) was repeated in the presence of the compound tamoxifen at a concentration of 1 nM (duration of preincubation with BM-531 was by a 10 min equilibration period).

Effects of OTBDS-estradiol-hexanoic acid *derivative on left ventricular pressure through adrenergic receptors*: Intracoronary boluses (50 µl) of the OTBDS-estradiol-hexanoic acid derivative (0.001 to 100 nM) were administered and the corresponding effect on the left ventricular pressure was determined. The dose-response curve (control) was repeated in the presence of prazosin or metoprolol at a concentration of 1 nM (duration of preincubation with prazosin or metoprolol was by a 10 min equilibration period).

Effect of OTBDS-estradiol-hexanoic acid *derivative on left ventricular pressure through synthesis of prostanglandins*: The boluses (50 µl) of the OTBDS-estradiol-hexanoic acid derivative [0.001 to 100 nM] were administered and the corresponding effect on the left ventricular pressure was evaluated. The bolus injection administered was done in the point of cannulation. The dose response curve (control) was repeated in the presence of indomethacin at a concentration of 1 nM (duration of the pre-incubation with indomethacin was for a period of 10 min).

Effects of OTBDS-estradiol-hexanoic acid *derivative on left ventricular pressure through the calcium channel activation:* Intracoronary boluses (50 µl) of the OTBDS-estradiol-hexanoic acid derivative [0.001 to 100 nM] were administered and the corresponding effect on the left ventricular pressure was evaluated. The dose-response curve (control) was repeated in the presence of nifedipine at a concentration of 1 nM (duration of the pre-incubation with nifedipine was for a period of 10 min).

Evaluation of effect induced by the OTBDS-estradiol-hexanoic acid *derivative on the intracellular calcium concentration:* Changes in intracellular calcium as a consequence of increases in time (3 to 18 min) in absence (control) and presence of OTBDS-estradiol-hexanoic acid derivative a concentration of 0.001 nM were determined using previously reports [14].

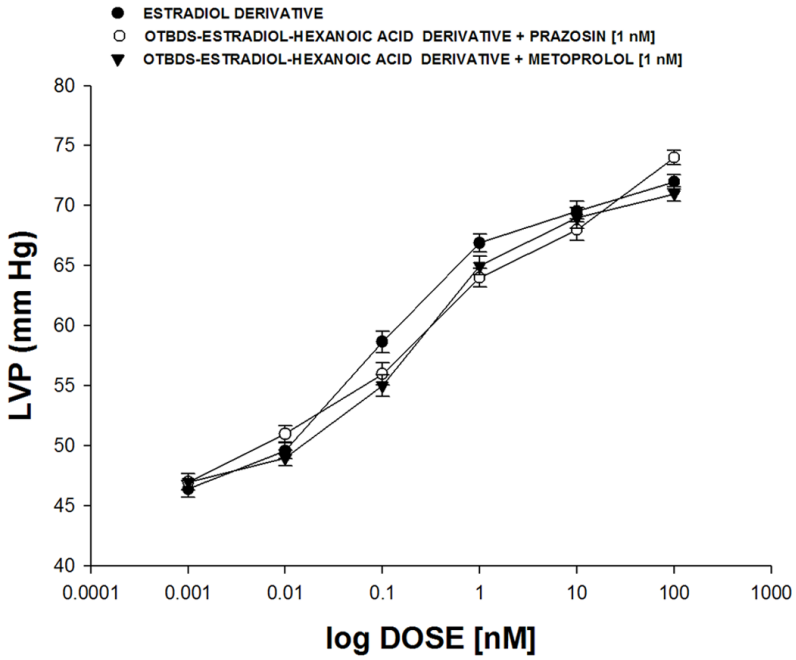

Figure 5. *Activity exerted by OTBDS-estradiol-hexanoic acid derivative on LVP through adrenergic receptors*. OTBDS-estradiol-hexanoic acid derivative [0.001 to 100 nM] was administered (intracoronary boluses, 50 µl) and the corresponding effect on the LVP was evaluated in the absence and presence of prazosin or metoprolol. The results showed that activity induced by the OTBDS-estradiol-hexanoic acid derivative on LVP was not inhibited in the presence of prazosin or metoprolol. Each bar represents the mean ± S.E. of 9 experiments. LVP = left ventricular pressure.

The effects were obtained in isolated hearts perfused at a constant-flow rate of 10 ml/min.

Statistical analysis

The obtained values are expressed as average ± SE, using each heart (n = 9) as its own control. The data obtained were put under Analysis of Variance (ANOVA) with the Bonferroni correction factor [15] using the SPSS 12.0 program. The differences were considered significant when *P* was equal or smaller than 0.05.

Results

Chemical synthesis

The yield of the reaction product (OTBDS-estradiol derivative, **Figure 1**) was 88% with melting point of 146-148°C. In addition, the chemical shifts of the spectroscopic analyses of ^{1}H NMR and ^{13}C NMR for the OTBDS-estradiol derivative is showed in the **Tables 1** and **2**. Finally, the results of mass spectroscopy (MS) (70 electrovolts) shown; m/z 500.30. Additionally, the elementary analysis data for the estradiol-OTBDS derivative ($C_{30}H_{52}O_2Si_2$) were calculated (C, 71.93; H, 10.46; O, 6.39; Si, 11.21) and found (C, 71.90; H, 10.44).

Other data showed a yield of 64% for the OTBDS-estradiol-hexanoic acid derivative (**Figure 1**) with melting point of 108-110°C. ^{1}H NMR and ^{13}C NMR the OTBDS-estradiol-hexanoic acid derivative is showed in the **Tables 3** and **4**. Finally, the results of mass spectroscopy (MS) (70 electrovolts) shown; m/z 643.40. Additionally, the elementary analysis data for the OTBDS-estradiol-hexanoic acid derivative ($C_{37}H_{65}NO_4Si_2$) were calculated (C, 69.00; H, 10.17; N, 2.17, O, 9.49; Si, 8.72) and found (C, 69.00; H, 10.14).

Biological activity

First stage

The activity induced by estradiol and its derivatives on perfusion pressure and coronary resistance in the isolated rat hearts was evaluated. The results obtained from changes in perfusion pressure as a consequence of increases in the time (3 to 18 min) in absence (control) or in presence of estradiol and its derivatives (**Figure 2**), showed that OTBDS-estradiol-hexanoic acid derivative [0.001 nM] significantly increase the perfusion pressure (*P* = 0.06) in comparison with estradiol, the OTBDS-estradiol derivative and control conditions [0.001 nM]. Other data (**Figure 3**) showed that coronary resistance, calculated as the ratio of perfusion pressure at coronary flow assayed (10 ml/min) was significantly higher (*P* = 0.05) in presence of the OTBDS-estradiol-hexanoic acid derivative at a concentration of 0.001 nM in comparison with estradiol, the OTBDS-estradiol derivative [0.001 nM] and control conditions.

Second stage

Other results showed that activity exerted by the OTBDS-estradiol-hexanoic acid derivative in dose of 0.001 to 100 nM increased the left

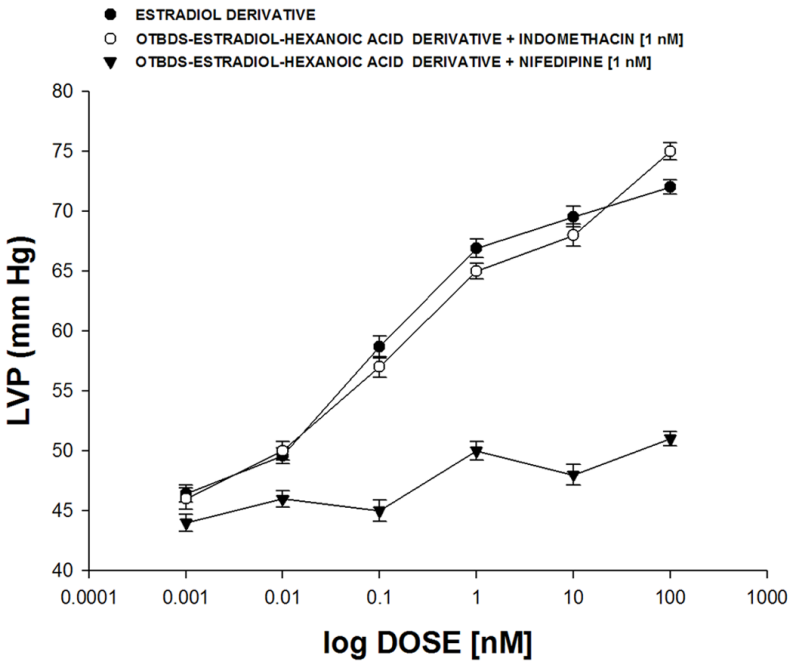

Figure 6. *Effects of OTBDS-estradiol-hexanoic acid derivative on LVP through prostaglandins synthesis*. Intracoronary boluses (50 µl) of the OTBDS-estradiol-hexanoic acid derivative [0.001 to 100 nM] were administered and the corresponding effect on the LVP was determined in the absence and presence of indomethacin. The results showed that the OTBDS-estradiol-hexanoic acid derivative increase the LVP in a dependent dose manner and this effect was not inhibited in the presence of indomethacin. Each bar represents the mean ± S.E. of 9 experiments. LVP = left ventricular pressure.

ventricular pressure (**Figure 4**) and this effect was not inhibited in presence of tamoxifen. In addition, other data indicate that pharmacological activity exerted by the OTBDS-estradiol-hexanoic acid derivative in dose of 0.001 to 100 nM was not inhibited by prazosin, metoprolol (**Figure 5**) or indomethacin (**Figure 6**) drugs at a concentration of 1 nM. In addition, in the **Figure 6** is showed the effect induced by the OTBDS-estradiol-hexanoic acid derivative on left ventricular pressure in a dose dependent manner [0.001 to 100 nM] was significantly inhibited by nifedipine ($P = 0.05$).

Finally, other results (**Table 5**) showed that the OTBDS-estradiol-hexanoic acid derivative at dose of 0.001 nM increase the intracellular calcium levels as a consequence of changes in the time (3 to 18 min) and this effect was partially inhibited with nifedipine.

Discussion

Chemical synthesis

In this study we report a straight forward route for the preparation of a two estradiol derivatives. In the first stage was synthesized the OTBDS-estradiol derivative using a reported previously method for protection of hydroxyl groups [16]. The structure of the OTBDS-estradiol derivative was confirmed using NMR spectroscopy (**Tables 1** and **2**). The ^{1}H NMR spectrum of the OTBDS-estradiol derivative shows signals at 0.06, 0.26, 0.88 and 1.06 ppm for methyl groups involved in the chemical structure of tert-buthyldimethylsilane group; at 0.84 ppm for methyl group bound to steroid nucleus; at 1.02 and 1.10-7.20 ppm for steroid moiety. The ^{13}C NMR spectra displays che-mical shifts at -4.5, -4.39, 18.00-18.10, 25.22 and 25.60 ppm for carbons involved in the chemical structure of tert-buthyldimethylsilane group; at 23.60 and 26.29-153.28 ppm for steroid moiety. Finally, the presence of the OTBDS-estradiol derivative was further confirmed from mass spectrum which showed a molecular ion at m/z 500.30.

The second stage was achieved by reaction of the OTBDS-estradiol derivative with aminocaproic acid to form the OTBDS-estradiol derivative-hexanoic acid derivative in presence of formaldehyde using Mannich method [17]. The ^{1}H NMR spectrum of the OTBDS-estradiol-hexanoic acid derivative showed signals at 0.06, 0.26 and 0.88-0.98 ppm for methyl groups involved in the chemical structure of tert-buthyldimethylsilane groups; at 0.84 ppm for methyl group bound to steroid nucleus; at 1.02-1.22, 1.34-1.38, 1.56-1.60, 1.70-2.10, 2.44-2.54, 3.50 and 6.60-6.80 ppm for steroid moiety; at 1.26, 1.48, 1.64, 2.40 and 2.72 ppm for methylene bound to both amine and carboxyl groups; at 3.64 ppm for methylene group bound to both ring A of steroid and amino group. The ^{13}C NMR spectra displays chemical shifts at -4.39, 18.00, 18.40 and 25.50-25.70 ppm for carbons involved in the chemical struc-

Table 5. Effect of the OTBDS-estradiol-hexanoic acid derivative [0.001 nM] on the intracellular calcium levels

Time (minutes)	Ca_i^{++} [mM]	
	Control	OTBDS-estradiol-hexanoic acid derivative
3	1.30×10^{-3}	6.75×10^{-4}
6	1.30×10^{-3}	6.75×10^{-4}
9	2.03×10^{-3}	6.75×10^{-4}
12	2.03×10^{-3}	6.75×10^{-4}
15	2.03×10^{-3}	6.75×10^{-4}
18	2.03×10^{-3}	6.75×10^{-4}

ture of tert-buthyldimethylsilane groups; at 11.30 ppm for methyl group bound to steroid nucleus; at 23.60, 26.10, 27.60-31.05, 38.40-44.50 and 51.10-151.00 for steroid moiety; at 25.22, 25.40, 27.38, 33.50 and 50.00 ppm for methylene groups bound to both carboxyl and amine groups; at 175.28 ppm for carboxyl group. Finally, the presence of the OTBDS-estradiol derivative-hexanoic acid derivative was further confirmed from mass spectrum which showed a molecular ion at m/z 643.40.

Biological evaluation

There are several reports which indicate that estradiol and some derivatives exert effects on cardiovascular system [18-20]; however there are not insufficient data on the inotropic activity exerted by estradiol derivatives. Therefore, in this study the effect exerted by a new estradiol derivative on blood vessel capacity and coronary resistance; translated as changes in perfusion pressure was evaluated in an isolated rat heart model. The results obtained show that the OTBDS-estradiol-hexanoic acid derivative significantly increases the perfusion pressure over time compared to estradiol, the OTBDS-estradiol derivative and the control conditions. To evaluate whether the activity induced by the OTBDS-estradiol-hexanoic acid derivative could depend of chemical structure the OTBDS-estradiol derivative was used as tool pharmacological. The results indicate that the OTBDS-estradiol derivative have not effects on perfusion pressure; these data indicate that hexanoic acid fragment bound to steroid nucleus is the responsible of activity of the OTBDS-estradiol-hexanoic acid derivative on perfusion pressure. To asses this hypotheses, the effect of the hexanoic acid was asses; the results showed that this compound have not activity on perfusion pressure (data not showed). These data indicate that only when hexanoic is bound to OTBDS-estradiol derivative have activity on perfusion pressure. This phenomenon, indicate that the OTBDS-estradiol-hexanoic acid derivative exerts effects on perfusion pressure which could subsequently modify vascular tone and coronary resistance of heart. In order to asses this hypothesis, the activity exerted by the OTBDS-estradiol-hexanoic acid derivative on coronary resistance was evaluated. The results indicate that coronary resistance was increased in presence of the OTBDS-estradiol-hexanoic acid derivative in comparison with the OTBDS-estradiol derivative and the control conditions.

Analyzing these results in this study was characterized the molecular mechanism by which the OTBDS-estradiol-hexanoic acid derivative exert its effect on blood pressure. To evaluate this phenomenon, several reports were analyzed which indicate that estradiol and some estradiol derivatives induces its effect via estrogen receptor [21, 22]. For this reason, we used tamoxifen an estrogen receptor blocker[8] to determine whether the effects of the OTBDS-estradiol-hexanoic acid derivative on left ventricular pressure were via the estrogen receptor. The results showed that effect exerted by the OTBDS-estradiol-hexanoic acid derivative on left ventricular pressure was not inhibited in the presence of tamoxifen which indicate that activity exerted by the estradiol derivative on left ventricular pressure was not via activation estrogen-receptor. Analyzing the data obtained and some reports which indicate that estrogen have effects on cardiovascular system through of the release or activation of vasoactive substances such as catecholamines which may induce a positive inotropic effect [23, 24]. In this study the effect exerted by the OTBDS-estradiol-hexanoic acid derivative on left ventricular pressure was evaluated in absence or presence of prazosin and metoprolol. The results showed that, the effect induced by the OTBDS-estradiol-hexanoic acid derivative on left ventricular pressure was not inhibited in presence of these compounds. These data indicate that the molecular mechanism involved in the effects of this estradiol-derivative on left ventricular pressure was not through adrenergic activity.

Therefore in search of molecular mechanism involved in the activity exerted by the OTBDS-estradiol-hexanoic acid derivative on left ventricular pressure, we also considered validating the activity induced by the OTBDS-estradiol-hexanoic acid derivative on stimulation and secretion of prostaglandins such happening with other type of steroid derivatives [25]. In this sense, in this experimental study, the activity exerted by the OTBDS-estradiol-hexanoic acid derivative on left ventricular pressure in the absence or presence of indomethacin was evaluated. The results showed that effect induced by the OTBDS-estradiol-hexanoic acid derivative on left ventricular pressure was not blocked by indomethacin. These data indicate that activity exerted by this steroid derivative on left ventricular pressure was not via prostanoids synthesis and secretion.

Therefore, analyzing the possibility of that OTBDS-estradiol-hexanoic acid derivative could induce its activity on left ventricular pressure through of activation of other molecular system that involved increase in the intracellular calcium and consequently bring a positive inotropic effect such as happening with other type of steroid derivative [26]. In this study, the activity induced by the OTBDS-estradiol-hexanoic acid derivative on left ventricular pressure was evaluated in the absence or presence of nifedipine. The results showed that effect exerted by the OTBDS-estradiol-hexanoic acid derivative was inhibited in the presence of nifedipine which indicate that activity exerted by this estradiol derivative involves activation calcium channel, this phenomenon can consequently bring an increase in the intracellular calcium concentration. To asses this hypotheses, in this study other experiments were carried out to evaluate the activity exerted by OTBDS-estradiol-hexanoic acid derivative on the concentration of intracellular calcium. The results indicate that the OTBDS-estradiol-hexanoic acid derivative increases the intracellular calcium levels as a consequence of changes in the time and this effect was inhibited with nifedipine. This data corroborates that the effect induced by the OTBDS-estradiol-hexanoic acid derivative indirectly affects the concentration of the intracellular calcium which is involved in changes in intraventricular pressure.

In conclusion, OTBDS-estradiol-hexanoic acid derivative is a particularly interesting drug because the positive inotropic activity induced by this compound involves L-type calcium channel activation. This phenomenon may result in a decrease in adverse effects such as cardiac arrhythmia and ischaemia induced by several cardiotonic agents such as cardiac glycosides and sympathomimetic amine.

Disclosure of conflict of interest

None.

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