CHANGES INDUCED BY ESTRADIOL-ETHYLENEDIAMINE DERIVATIVE ON PERFUSION PRESSURE AND CORONARY RESISTANCE IN ISOLATED RAT HEART: L-TYPE CALCIUM CHANNEL

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Aim. The present study was designed to investigate the effects of estradiol-ethylenediamine derivative on perfusion pressure and coronary resistance in rats. An additional aim was to identify the molecular mechanisms involved.

Methods. The Langendorff model was used to measure perfusion pressure and coronary resistance changes in isolated rat heart after estradiol-ethylenediamine derivative alone and following compounds; tamoxifen (estrogen receptor antagonist), prazosin (alpha 1 adrenoreceptor antagonist), metoprolol (selective beta 1 receptor blocker), indomethacin (prostaglandin synthesis inhibitor) and nifedipine (L-type calcium-channel inhibitor).

Results. The results show that estradiol-ethylenediamine derivative [10^{-9} mmol] significantly increased perfusion pressure ($p = 0.005$) and coronary resistance ($p = 0.006$) in isolated rat heart. Additionally, the effect of estradiol-ethylenediamine on perfusion pressure [10^{-9} to 10^{-4} mmol] was only blocked in the presence of the L-type calcium-channel (nifedipine).

Conclusions. These data suggest that the effect of estradiol-ethylenediamine on perfusion pressure and vascular coronary involves activation of the L-type calcium channel through a non-genomic molecular mechanism.

INTRODUCTION

High blood pressure contributes substantially to cardiovascular disease incidence and premature mortality^{1–3}. Studies monitoring ambulatory blood pressure have shown that blood pressure is higher in men than in women of similar age^{4,5}. In addition, experimental and clinical studies^{6–8} have demonstrated that sex hormones may be associated with hypertension. Further, sex differences in blood pressure regulation in humans have also been demonstrated in various animal models^{9–11}. For example, male spontaneously hypertensive rats (SHR) have higher blood pressure than do females of similar age^{12–14}. These data suggest that among others, estrogen levels may influence blood pressure. In this regard, there is evidence that estrogen replacement in ovariectomized rats reduces arterial pressure response to psychological stress and that these effects are mediated, at least in part by nitric oxide¹⁵. In addition, estrogens have been shown to protect transgenic hypertensive rats by shifting the vasoconstrictor-vasodilator balance of the renin-angiotensin system and consequently induce change in blood pressure¹⁶.

There are also studies which suggest that some estradiol derivatives also induce changes in blood pressure as well; for example, Bonacasa et al¹⁷, reported indicate

that estradiol derivative (2-methoxyestradiol) attenuates hypertension in spontaneously hypertensive rats while 2-hydroxyestradiol was found to reduce blood pressure in an obese rat model¹⁸. The molecular mechanism of these two estradiol derivatives involves inhibition of endothelin-1 synthesis by means of an estrogen receptor-independent mechanism¹⁹. These data taken together show that estradiol and its derivatives induce changes in blood pressure; nevertheless, the cellular site and molecular mechanisms involved are very confusing. Differences in the chemical structure of estradiol and its derivatives may be in part responsible. To test this, 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 model. To evaluate the molecular mechanisms involved, the following compounds were used as pharmacological tools for blocking various receptors; tamoxifen (estrogen receptor antagonist) (ref.²⁰), prazosin (α_1 adrenoreceptor antagonist) (ref.²¹), metoprolol (selective β_1 receptor blocker) (ref.²²), indomethacin (prostaglandin synthesis inhibitor) (ref.²³) and nifedipine (L-type calcium-channel inhibitor) (ref.²⁴).

MATERIAL AND METHODS

General methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of Universidad Autonoma de Campeche (UAC) and were in accordance with the Guide for the Care and Use of Laboratory Animals

(Washington, DC: National Academy Press, 1996) (ref.²⁵). Female rats (Wistar; weighing 200–250 g) were obtained from UAC.

Reagents

Estradiol-ethylenediamine derivative (Fig. 1) was prepared according to a previously reported method by Figueroa et al.²⁶. Other reagents were obtained from Sigma-Aldrich Chemical Co. All drugs were dissolved in *methanol* and different dilutions were obtained using Krebs-Henseleit solution ($\leq 0.01\%$, v/v).

Langendorff method.

Briefly, the female rat (200–250 g) was anesthetized by injection with pentobarbital at a dose rate of 50 mg/kg body weight. The chest was then opened, and a loose ligature passed through the ascending aorta. The heart was rapidly removed and immersed in ice cold physiological saline solution. It 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 CaCl₂; 1.2 NaH₂PO₄; 1.2 MgSO₄; 24.2 NaHCO₃; 5 glucose, and 5 sodium pyruvate. The solution was actively bubbled with a mixture of O₂/CO₂ (95:5). The coronary flow was adjusted with a variable-speed peristaltic pump. An initial perfusion rate of 15 ml/min for 5 min was followed by a 25 min equilibration period at a perfusion rate of 10 ml/min. All experimental measurements were made after the equilibration period.

Perfusion pressure

Evaluations of perfusion pressure changes due to drugs were made using a pressure transducer connected to

the chamber where the hearts were mounted. The results were entered into a computerized data capture system (Biopac).

Experimental design

Eighteen animals were used, 9 for both control and treatment groups. The control group received no drug while the treatment group functioned as its own control

Effect of estradiol-ethylenediamine derivative on perfusion pressure. Changes in perfusion pressure as a consequence of increase in time (3–18 min) in the absence (control) or presence of estradiol-ethylenediamine derivative at a concentration of 10⁻⁹ mmol were determined. The effects were obtained in isolated hearts perfused at a constant-flow rate of 10 ml/min.

Effects of estradiol-ethylenediamine derivative on coronary resistance. Coronary resistance in the absence (control) or presence of estradiol-ethylenediamine derivative at a concentration of 10⁻⁹ mmol was evaluated. The effects were obtained in isolated hearts perfused at a constant flow rate of 10 ml/min. The coronary resistance was determined as the relationship between coronary flow and perfusion pressure (mm Hg/ml/min).

Effects of estradiol-ethylenediamine derivative on perfusion pressure through the estrogen receptors. Intracoronary boluses (50 μ l) of estradiol-ethylenediamine derivative [10⁻⁹ to 10⁻⁴ mmol] were administered and the corresponding effect on perfusion pressure was determined. The dose-response curve (control) was re-determined in the presence of tamoxifen at a concentration of 10⁻⁶ mmol (duration of preincubation with tamoxifen was by a 10 min equilibration period).

Effect of estradiol-ethylenediamine derivative on perfusion pressure through the α_1 adrenergic receptor. Intracoronary boluses (50 μ l) of estradiol-ethylenediamine derivative [10⁻⁹ to 10⁻⁴ mmol] were administered and the corresponding effect on perfusion pressure was evaluated. The dose-response curve (control) was determined in the presence of prazosin at a concentration of 10⁻⁶ mmol (duration of preincubation with prazosin was a 10 min equilibration period).

Effects of estradiol-ethylenediamine derivative on perfusion pressure through the β_1 adrenergic receptor. Intracoronary boluses (50 μ l) of estradiol-ethylenediamine derivative [10⁻⁹ to 10⁻⁴ mmol] were administered and the corresponding effect on the perfusion pressure was evaluated. The dose-response curve (control) was again obtained in the presence of metoprolol at concentration of 10⁻⁶ mmol (duration of preincubation with metoprolol was by a 10 min equilibration period).

Changes induced by estradiol-ethylenediamine derivative on perfusion pressure through synthesis of prostaglandins. Intracoronary boluses (50 μ l) of estradiol-ethylenediamine derivative (10⁻⁹ to 10⁻⁴ mmol) were administered and the corresponding effect on the perfusion pressure was evaluated. The dose-response curve (control) was determined in the presence of indomethacin at a concentration of 10⁻⁶ mmol (duration of preincubation with indomethacin was a 10 min equilibration period).

Fig. 1. Chemical structure of estradiol derivative.

Fig. 2. Effect induced by *estradiol* derivative on perfusion pressure. The results show that *estradiol derivative* [10⁻⁹ mmol] significantly increased perfusion pressure ($p = 0.005$) through time (3–18 min) in comparison with the control conditions. Each bar represents the mean $\pm$ S.E. of 9 experiments.

Fig. 3. Activity exerted by *estradiol derivative* on coronary resistance. The results show that coronary resistance was higher ($p = 0.006$) in the presence of *estradiol derivative* [10⁻⁹ mmol] in comparison with the control conditions. Each bar represents the mean $\pm$ S.E. of 9 experiments.

Fig. 4. Effects induced by *estradiol derivative* on perfusion pressure through estrogen receptors. Intracoronary boluses (50 μ l) of *estradiol derivative* [10⁻⁹ to 10⁻⁴ mmol] were administered and the corresponding effect on the perfusion pressure was determined. The results showed that *estradiol derivative* increase the perfusion pressure in a dependent dose manner and this effect was not inhibited in presence of *tamoxifen* [10⁻⁶ mmol]. Each bar represents the mean $\pm$ S.E. of 9 experiments.

Fig. 5. Effect exerted by *estradiol derivative* on perfusion pressure through of α_1 adrenergic receptor. *Estradiol derivative* [10⁻⁹ to 10⁻⁴ mmol] was administered (intracoronary boluses, 50 μ l) and the corresponding effect on the perfusion pressure was evaluated in absence and presence of *prazosin* [10⁻⁶ mmol]. The results showed that activity induced by *estradiol derivative* on perfusion pressure was not inhibited in presence of *prazosin*. Each bar represents the mean $\pm$ S.E. of 9 experiments.

Effects of estradiol-ethylenediamine derivative on perfusion pressure through the calcium channel. Intracoronary boluses (50 μ l) of estradiol-ethylenediamine derivative [10⁻⁹ to 10⁻⁴ mmol] were administered and the corresponding effect on the perfusion pressure was evaluated.

The dose-response curve (control) was obtained in the presence of nifedipine at a concentration of 10⁻⁶ mmol (duration of preincubation with nifedipine was a 10 min equilibration period).

Fig. 6. Activity induced by estradiol derivative on perfusion pressure through of β_1 -adrenergic receptor. Intracoronary boluses (50 μ l) of estradiol derivative [10^{-9} to 10^{-4} mmol] were administered and the corresponding effect on the perfusion pressure was evaluated in absence and presence of metoprolol (10^{-6} mmol). The results showed that activity induced by estradiol derivative on perfusion pressure was not inhibited in presence of metoprolol. Each bar represents the mean $\pm$ S.E. of 9 experiments.

Fig. 7. Effects induced by estradiol derivative on perfusion pressure through of synthesis of prostaglandins. Intracoronary boluses (50 μ l) of estradiol derivative [10^{-9} to 10^{-4} mmol] were administered in absence and presence of indomethacin [10^{-6} mmol]. The results showed that effect induced by estradiol derivative on perfusion pressure in presence of indomethacin was not inhibited. Each bar represents the mean $\pm$ SE of 9 experiments.

Statistical analysis

The results are expressed as means $\pm$ SE, using each heart as its own control. The data were analyzed using analysis of variance repeated measures (ANOVA) and the Bonferroni correction factor²⁷. The differences were considered significant when p was equal to or smaller than 0.05.

RESULTS

Changes in perfusion pressure as a consequence of increase in time (3–18 min) in the absence (control) or presence of estradiol-ethylenediamine derivative (Fig. 2) were evaluated. The results showed that estradiol derivative [10^{-9} mmol] significantly increased the perfusion pressure ($p = 0.005$) in comparison with the control. (Fig. 3) shows that coronary resistance calculated as the ratio of perfusion pressure at the coronary flow assayed (10 ml/min) was higher ($p = 0.006$) in the presence of estradiol-ethylenediamine derivative [10^{-9} mmol] than in controls. (Fig. 4) shows that estradiol-ethylenediamine derivative, increased the perfusion pressure in a dose dependent manner [10^{-9} to 10^{-4} mmol] and that this effect was not inhibited by tamoxifen [10^{-6} mmol].

The effect of estradiol-ethylenediamine derivative [10^{-9} to 10^{-4} mmol] on perfusion pressure in the presence of prazosin (Fig. 5) or metoprolol (Fig. 6) at a concentration of 10^{-6} mmol was not inhibited. (Fig. 7) shows that

Fig. 8. Activity exerted by estradiol derivative on perfusion pressure through L-type calcium channel. Intracoronary boluses (50 μ l) of estradiol derivative [10^{-9} to 10^{-4} mmol] were administered in absence and presence of nifedipine [10^{-6} mmol]. The results showed that effect induced by estradiol derivative on perfusion pressure in presence of nifedipine was inhibited significantly ($p = 0.005$). Each bar represents the mean $\pm$ SE of 9 experiments.

the effect of estradiol-ethylenediamine derivative [10^{-9} to 10^{-4} mmol] on perfusion pressure was not blocked by indomethacin [10^{-6} mmol]. Finally, the effect of estradiol-ethylenediamine derivative [10^{-9} to 10^{-4} mmol] on perfusion pressure in the presence of nifedipine (Fig. 8) was significantly inhibited ($p = 0.005$).

DISCUSSION

In this study, we evaluated the effect of estradiol-ethylenediamine derivative on blood vessel capacity and coronary resistance translated as changes in perfusion pressure in isolated rat heart (Langendorff model). The results show that estradiol-ethylenediamine derivative significantly increased perfusion pressure over time (3–18 min) compared to the controls. These data suggest that estradiol-ethylenediamine derivative exerts effects on perfusion pressure, which could subsequently modify vascular tone and coronary resistance. To test this hypothesis, we evaluated the effects induced by estradiol-ethylenediamine derivative on coronary resistance. We found that coronary resistance was increased by the estradiol-ethylenediamine derivative. These data suggest that estradiol-ethylenediamine derivative exerts effect on vascular tone.

To characterize the molecular mechanism of this phenomenon we noted the reports of some investigations^{28–30} which indicate that estradiol induces its effect on blood pressure via activation of the estrogen receptor. For this reason, we used tamoxifen, an estrogen receptor blocker to determine if the effects of estradiol-ethylenediamine derivative on perfusion pressure were via the estrogen receptor. Interaction of estradiol-ethylenediamine derivative with the estrogen-receptor, may be a key requirement for the biological activity as in the case of other estradiol derivatives^{31,32}. Our results showed that the effects of estradiol-ethylenediamine derivative were not inhibited by tamoxifen, suggesting that the molecular mechanism is not via the estrogen-receptor.

On the other hand, the molecular mechanism proposed by some investigators³³ suggests that estradiol can exert an indirect tonic effect on adrenal catecholamine synthesis. Some studies report that estradiol decreases norepinephrine levels and consequently induces low blood pressure^{34,35}. To evaluate the biological activity of estradiol-ethylenediamine derivative on perfusion pressure in the absence or presence of prazosin (α_1 adrenoreceptor antagonist) and metoprolol (selective β_1 receptor blocker) was evaluated. Our results showed that the effect of estradiol-ethylenediamine derivative was not inhibited by these compounds, indicating that the molecular mechanism involved is not through adrenergic activity.

Some steroids³⁶ have been found to exert effects on perfusion pressure via-prostaglandins stimulation and secretion. For this reason we used the prostaglandins synthesis inhibitor indomethacin to test this concept. The results showed that indomethacin did not block the effects of estradiol-ethylenediamine derivative, suggesting that mechanism was not via prostaglandins synthesis and secretion. Analyzing these data and reports indicat-

ing that the effect of a number of steroids on blood pressure involves activation of the calcium channel³⁷, we used nifedipine an *L*-type calcium channel inhibitor. The results showed that the activity of estradiol-ethylenediamine derivative was significantly blocked by nifedipine.

The results suggest that the effect of estradiol derivative on perfusion pressure may be to increase calcium levels through activation of the *L*-type calcium channel via a non-genomic molecular mechanism. This explanation is similar to explanations for the effect of some steroid derivatives on perfusion pressure involving increase in intracellular calcium through a non-genomic mechanism^{38,39}.

CONCLUSIONS

Overall, the results suggest that the effect of estradiol-ethylenediamine derivative on perfusion pressure and coronary resistance is dependent on its chemical structure and may involve the *L*-type calcium channel activation through a non-genomic molecular mechanism.

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