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Activity induced by a danazol derivative on perfusion pressure and coronary resistance in isolated rat heart

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Abstract

Experimental studies suggest that danazol can be associated with changes in blood pressure. Nevertheless, there is scarce information about the effects of danazol and its derivatives at cardiovascular level. To clarify on this phenomenon, we evaluated the effects of danazol derivative on perfusion pressure in isolated rat heart using Langendorff flow model. Our results demonstrated that pregnenolone-derivative (10^{-9} mM) significantly increase the perfusion pressure ($p = 0.05$) and vascular resistance ($p = 0.05$) in isolated heart. The activity induced by danazol derivative on perfusion pressure (10^{-9} to 10^{-4} mM) was blocked in presence of nifedipine (10^{-6} mM).These data suggest that activity induced by danazol derivative on perfusion pressure and vascular resistance is dependent upon its chemical structure. This phenomenon involves the L-type calcium channel activation through a non-genomic molecular mechanism.

Key words: Danazol, derivative, Langendorff, perfusion pressure.

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Introduction

High blood pressure contributes substantially to cardiovascular disease incidence and premature mortality [1,2]. Studies using the technique of ambulatory blood pressure monitoring have shown that blood pressure is higher in men than in women of similar ages [3,4]. Experimental and clinical studies [5-7] has demonstrated that androgens can be associated with hypertension development. In particular**,** there are some reports which indicate that danazol is associated with hypertension [8]. For example the studies reported by Wu and coworkers [9] who suggest that there is a relationship between the danazol and hypertension. These data are supported by the studies of Bretza and coworkers [10]. which indicate that administration of danazol increase the blood pressure, this phenomenon was associated with changes in the sodium levels. Nevertheless, it is important to mention that other clinical data show that activity induced by danazol could be an independent factor of hypertension associated to arterial thrombosis [11].

Recently, a study [12] showed that a danazol derivative (hemisuccinate of danazol) increases the perfusion pressure and resistance vascular in isolated rat heart via interaction of androgen-receptor.

Apart from the above experiments, which also do not show clearly the cellular site and actual molecular mechanisms of danazol and its derivatives, data information are needed for characterizing the activity induced by this steroid and its derivatives at cardiovascular level. To provide this information, the present study was designed to investigate the effects of danazol and the danazol derivative (PDE) on perfusion pressure and coronary resistance in isolated rat hearts using Langendonff model [13]. Additionally, the molecular mechanism involved in the activity induced by PDE on perfusion pressure was evaluated using several substances such as flutamide (androgen receptor antagonist) [14], prazosin (α_1 adrenoreceptor antagonist) [15], metoprolol (selective β_1 receptor blocker) [16] and nifedipine (antagonist calcium channel *type* L) [17] as pharmacological tools.

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 [18]. . Male rats (Wistar; weighing 200-250 g; n = 63) were obtained from UAC.

Reagents

Pregnenolone-danazol-ethylendiamine conjugate (succinic acid 6-[2-amino-ethylamino)-methyl]-2-ethynyl-10a,12a-di-methyl-2,3,3-3b-,5,10,10a,10b,11,12,12a, dodecahydro-1*H*-7-oxa-8-aza-dicyclopenta[*a,h*] phenanthren-1-yl-ester-17-acetyl,10,13-dimethyl-2,3,4,7,8,9, 10,11, 12, 13,14,15,16,17-tetradecahydro-1-*H*-cyclopenta [*a*] phenanthren- 3-yl ester) showed in Figure 1 was prepared according to a previously reported method by Figueroa and coworkers [19]. Other reagents were obtained from Sigma-Aldrich Chemical Co. All drugs were dissolved in methanol and different dilutions were obtained using Krebs-Henseleit solution (0.01%, v/v).

Langendorff method

Briefly, the male rat (200 - 250 g) was anesthetized by injecting them with pentobarbital at a dose of 50 mg/Kg body weight. 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 noncirculating perfusion system at a constant flow rate. The perfusion medium was the Krebs-Henseleit solution (pH 7.4, 37°C) composed of (mM); 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).

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 done within the equilibration period.

Perfusion pressure

Evaluations of perfusion pressure changes induced by drugs 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).

Biological evaluation

Effect induced by pregnenolone, danazol and PDE on perfusion pressure.

Time course changes in perfusion pressure of pregnenolone, danazol and PDE at a concentration of 10^{-9} mM were determined. The effects were obtained in isolated hearts perfused at a constant-flow rate of 10 ml/min. It is important to mention that conditions used were manly the previous reports [20].

Evaluation of effects exerted by pregnenolone, danazol and PDE on coronary resistance.

The coronary resistance in absence (control) or presence of pregnenolone, danazol and PDE at a concentration of 10^{-9} mM was evaluated. The effects were obtained in isolated hearts perfused at a constant flow rate of 10 ml/min. The coronary resistance was determined by the relationship between coronary flow and perfusion pressure (mm Hg/ml/min) [21,22].

Effects induced by PDE on perfusion pressure through androgen receptors.

Intracoronary boluses (50 µl) of PDE [10^{-9} to 10^{-4} mM] were administered and the corresponding effect on the perfusion pressure was determined. The dose-response curve (control) was repeated in the presence of flutamide at a concentration of 10^{-6} mM (duration of preincubation with flutamide was by a 10 min equilibration period). The dose of adrenergic antagonist was using previous reports[23]

Effect exerted by PDE on perfusion pressure in the presence of α_1 adrenergic blocker.

The boluses (50 µl) of PDE [10^{-9} to 10^{-4} mM] were administered and the corresponding effect on the perfusion pressure was evaluated. It is important to mention that the bolus injection administered was done in the point of cannulation. The dose-response curve (control) was repeated in the presence of prazosin at a concentration of 10^{-6} mM (duration of preincubation with prazosin was by a 10 min equilibration period). The dose of α_1 adrenergic antagonist was using the reports by Figueroa[23] Drew [24].

Effects induced by PDE on perfusion pressure in the presence of β_1 adrenergic blocker.

The boluses (50 µl) of PDE [10^{-9} to 10^{-4} mM] were administered and the corresponding effect on the perfusion pressure was evaluated. The dose-response curve (control) was repeated in the presence of metoprolol at concentration of 10^{-6} mM (duration of preincubation with metoprolol was by a 10 min equilibration period). The dose of β_1 adrenergic antagonist was using previous reports [21,22,25]..

Activities exerted by PDE on perfusion pressure in the presence of calcium channel blocker.

The boluses (50 µl) of PDE [10^{-9} to 10^{-4} mM] were administered and the corresponding effect on the perfusion pressure was evaluated. The dose-response curve (control) was repeated in the presence of nifedipine at a concentration of 10^{-6} mM (duration of preincubation with nifedipine was by a 10 min equilibration period). The dose of calcium antagonist was using previous reports [24].

Statistical analysis

The obtained values are expressed as mean ± SE (standard error), using each heart as its own control. The comparation between means was made with a paired Stu-

dent's t test. In the case multiple comparation was used an analysis of variance (ANOVA) using the Bonferroni correction factor[26]. The differences were considered significant when *p* was equal or smaller than 0.05.

Results

In this study, the activity induced by danazol, pregnenolone and pregnenolone-danazol-ethylendiamine conjugate (PDE) on perfusion pressure and coronary resistance in isolated rat heart was evaluated. The results obtained from changes in perfusion pressure as a consequence of increases in the time (3-18 min) in absence (control) or in presence of pregnenolone, danazol and PDE (Figure 2), showed that PDE [10^{-9} mM] significantly increase the perfusion pressure (***p*** = 0.05) in comparison with the control conditions, pregnenolone and danazol at the same dose. Additionally, another result showed that coronary resistance, calculated as the ratio of perfusion pressure at coronary flow assayed (10 ml/min) was higher in the presence of PDE than in control conditions, pregnenolone and danazol (***p*** = 0.05) at a concentration of 10^{-9} mmol (Figure 3). Another result (Figure 4) showed that PDE increase the perfusion pressure in a dose dependent manner [10^{-9} to 10^{-4} mM] and this effect was not inhibited in presence of flutamide [10^{-6} mM].

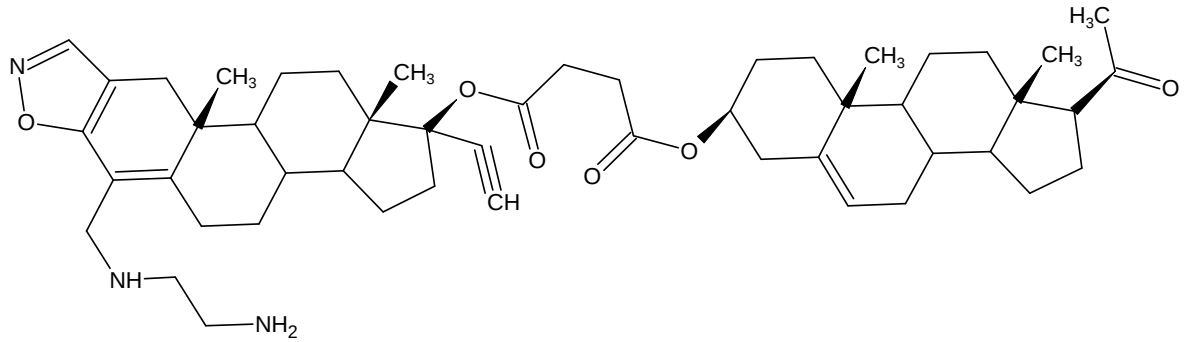

Figure 1. *Chemical structure of pregnenolone-danazol-ethylendiamine.*

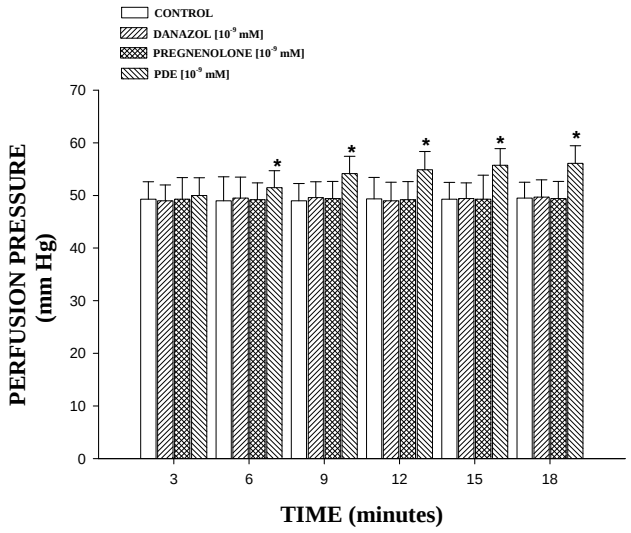

Figure 2. *Effect induced by pregnenolone, danazol and pregnenolone-danazol-ethylendiamine (PDE) on perfusion pressure. The results showed that PDE significantly increase the perfusion pressure (**p** = 0.05) through of time (3 - 18 min) in comparison with the control conditions, pregnenolone and danazol. Each bar represents the mean ± S.E. of 9 experiments.*

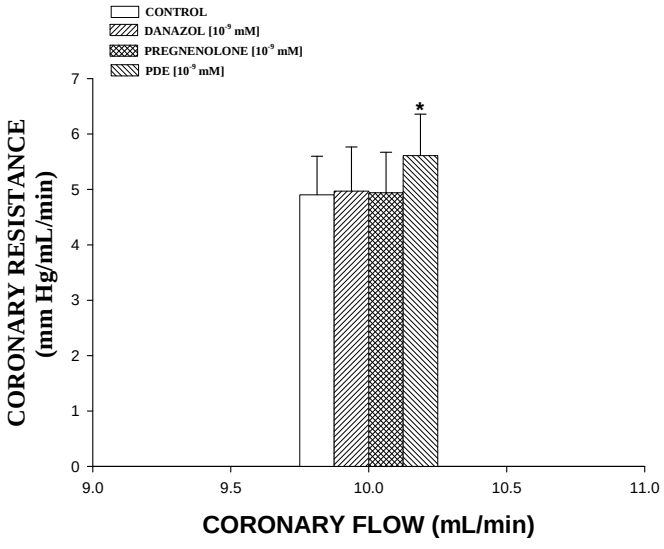

Figure 3. *Activity induced by pregnenolone-danazol-ethylendiamine (PDE) on coronary resistance. The results showed that coronary resistance was high (**p** = 0.05) in presence of PDE in comparison with the control conditions, pregnenolone and danazol. Each bar represents the mean ± S.E. of 9 experiments.*

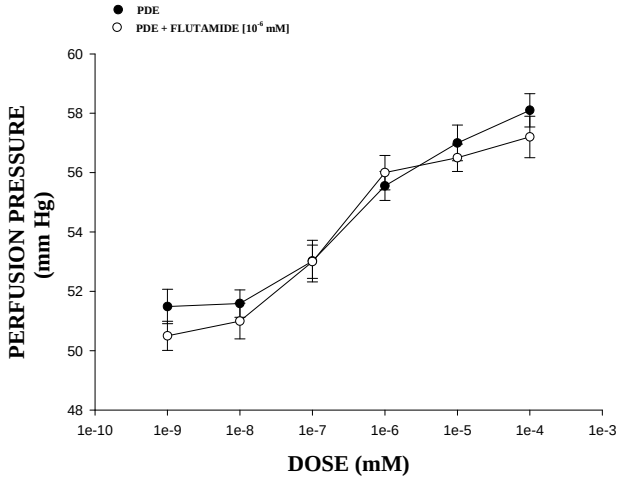

Figure 4. *Effects induced by pregnenolone-danazol-ethylendiamine (PDE) on perfusion pressure through androgen receptors. Intracoronary boluses (50 µl) of PDE [10^{-9} to 10^{-4} mM] were administered and the corresponding effect on the perfusion pressure was determined. The results showed that PDE increase the perfusion pressure in a dependent dose manner and this effect was not inhibited in presence of flutamide [10^{-6} mM]. Each bar represents the mean ± S.E. of 9 experiments.*

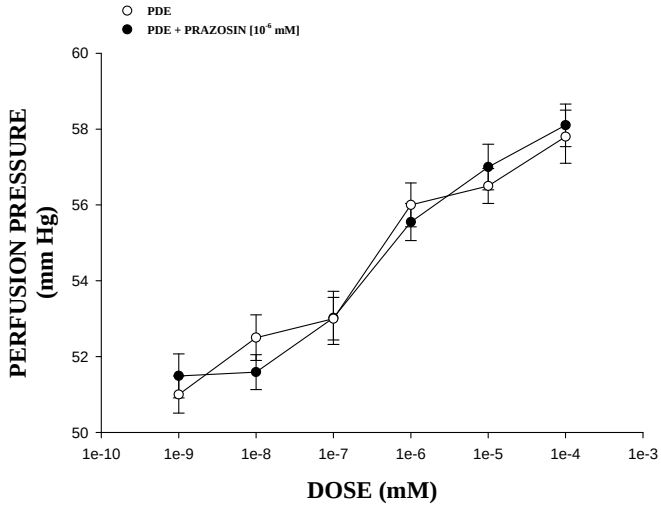

Figure 5. *Effect exerted by pregnenolone-danazol-ethylendiamine (PDE) on perfusion pressure through of α_1 adrenergic receptor. PDE [10^{-9} to 10^{-4} M] was administered (intracoronary boluses, 50 µl) and the corresponding effect on the perfusion pressure was evaluated in absence and presence of prazosin [10^{-6} mM]. The results showed that activity induced by PDE on perfusion pressure was not inhibited in presence of prazosin. Each bar represents the mean ± S.E. of 9 experiments*

On the other hand, other experiments showed that PDE increase the perfusion pressure in a dose dependent manner [10^{-9} to 10^{-4} mM] and this effect was not inhibited in

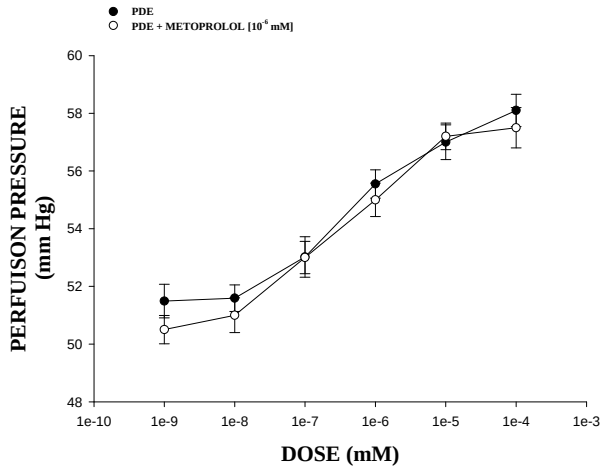

Figure 6. *Activity induced by pregnenolone-danazol-ethylendiamine (PDE) on perfusion pressure through of β_1- adrenergic receptor. Intracoronary boluses (50 µl) of PDE [10^{-9} to 10^{-4} mM] were administered and the corresponding effect on the perfusion pressure was evaluated in absence and presence of metoprolol [10^{-6} mM]. The results showed that activity induced by PDE on perfusion pressure was not inhibited in presence of metoprolol. Each bar represents the mean ± S.E. of 9 experiments*

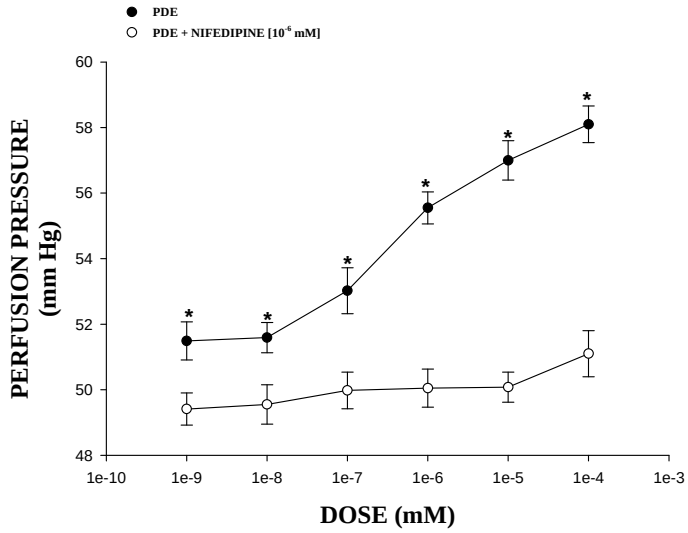

Figure 7. *Effects induced by pregnenolone-danazol-ethylendiamine (PDE) on perfusion pressure through L type calcium channel. The boluses (50 µl) of PDE [10^{-9} to 10^{-4} mM] were administered in absence and presence of nifedipine [10^{-6} mM].The results showed that effect induced by PDE on perfusion pressure in presence of nifedipine was inhibited significantly (**p** = 0.06). Each bar represents the mean ± SE of 9 experiments*

presence of prazosin (Figure 5) and metoprolol (Figure 6) at a concentration of 10^{-6} mM. Alternative experimental indicate that the activity exerted by PDE [10^{-9} to 10^{-4} mM] on perfusion pressure (Figure 7) in presence of nifedipine at a concentration of 10^{-6} mM was significantly inhibited ($p = 0.06$).

Discussion

In this study, was evaluated the effect of danazol derivative on blood vessel capacity and coronary resistance translated as changes in perfusion pressure in isolated rat heart (Langendorff model). The results show that danazol derivative significantly increased perfusion pressure over time (3-18 min) compared to the control conditions. It is important to mention that analyzing the possibility of that both danazol and pregnenolone fragments involved in the chemical structure of danazol derivative could be the responsible of the activity induced by this steroid derivative on the perfusion pressure, the effects exerted by pregnenolone and danazol on the perfusion pressure were evaluated to compare with the activity induced by danazol derivative. The results indicate that perfusion pressure was not affected in presence of both danazol and pregnenolone in comparison with the control conditions. Those experimental data indicate that the steroid derivative induce effects on perfusion pressure, which could consequently bring modifications in coronary resistance as it happens in another type of steroid derivatives[20-23]. To test this hypothesis, we evaluated the effects induced by danazol derivative on coronary resistance. We found that coronary resistance was increased by the danazol derivative in comparison with pregnenolone, danazol and control conditions. These data suggest that steroid derivative exerts effect on vascular tone. To characterize the molecular mechanism of this phenomenon we noted the reports of some investigations[27,28] which indicate that some androgens induce its effect on blood pressure via activation of the androgen receptor[27]. For this reason, we used flutamide (androgen receptor blocker) to determine if the effects of danazol derivative on perfusion pressure were via the androgen receptor activation as in the case of other androgen derivatives[21-23]. Our results showed that the effects of danazol derivative were not inhibited by flutamide, suggesting that the molecular mechanism is not via the androgen-receptor.

On the other hand, analyzing data obtained in this study and the report on the molecular mechanism proposed by Kumai and coworkers[28] which suggests that some steroids can exert an indirect tonic effect on adrenal catecholamine synthesis and secretion. In Addition, of other studies which suggest that androgens stimulate the increased expression of adrenergic receptors (in some cellular lines), which has an important role in the development or maintenance of elevated blood pressure[29]. To evaluate this hypothesis in this study, the effect exerted by danazol derivative on perfusion pressure was evaluated in absence or presence of prazosin (α_1 adrenoreceptor antagonist) and metoprolol (selective β_1 receptor blocker). Our results showed that the effect induced by the danazol derivative was not inhibited in presence of these compounds. These data indicate that molecular mechanism involved in the effects of this danazol derivative on perfusion pressure is not through adrenergic activity.

Therefore, analyzing experimental data obtained in this study, we also considered validating the effect induced by some steroids on perfusion pressure *via* calcium-channels[30]. To evaluate this hypothesis in this study, the effect induced by danazol derivative on perfusion pressure was evaluated in absence or presence nifedipine (antagonist *type* L calcium-channel). The results showed that activity of steroid derivative in presence of nifedipine was blocked significantly. These data are similar to some reports[23] which showed that the pressor effect of some steroid derivatives is partially reversed by the subsequent administration of nifedipine. In conclusion, the results obtained suggest that activity induced by danazol derivative on perfusion pressure and vascular resistance is dependent upon its chemical structure. This phenomenon involves activation of the *L-type calcium channel* via a non-genomic molecular mechanism

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