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Full Length Research Paper

Activity induced by *dihydrotestosterone-dihydropyrimidine derivative* on perfusion pressure and coronary resistance in isolated rat heart

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Experimental and clinical studies suggest that *dihydrotestosterone* can be associated with changes in blood pressure. In this work, the effects induced by *dihydrotestosterone* and d*ihydrotestosterone-dihydropyrimidine* on perfusion pressure and coronary resistance were evaluated, in isolated rat heart using the Langendorff flow model. Additionally, the molecular mechanism involved in the activity exerted by d*ihydrotestosterone-derivative* was characterized. The results showed that d*ihydrotestosterone-dihydropyrimidine* [10^{-9} mM] significantly increase the perfusion pressure (*p* = 0.005) and coronary resistance (*p* = 0.006) in isolated rat heart. Additionally, the activity exerted by d*ihydrotestosterone-dihydropyrimidine* on perfusion pressure [10^{9} to 10^{-4} mM] was blocked in the presence of *nefidepine* [10^{-6} mM].These data suggest that activity induced by d*ihydrotestosterone-derivative* on perfusion pressure and coronary resistance is dependent upon its chemical structure. This phenomenon possibly involves the *L-type calcium channel* activation through a non-genomic molecular mechanism.

Key words: *Dihydrotestosterone,* perfusion pressure, vascular resistance.

INTRODUCTION

High blood pressure contributes substantially to cardiovascular disease incidence and premature mortality (Stary, 1989; Mhoney et al., 1996; Oparil et al., 2003). Studies using the technique of ambulatory blood pressure monitoring have shown that blood pressure is higher in men than in women of similar ages (Khoury et al., 1992; Wiinberg et al., 1995). In addition, experimental and clinical studies (Khaw and Barret, 1988; Gray et al., 1991a; Gray et al., 1991b) have demonstrated that sex hormones can be associated with hypertension development. It is important to mention that the sex-associated differences in blood pressure regulation observed in humans have also been documented in various animal models. For example, male spontaneously hypertensive rats (SHR) have higher blood pressures than do females of similar ages (Masubuchi et al., 1982; Cheng and Meng, 1991; Reckelhoff et al., 1998).

Although, the mechanisms responsible for the gender differences in blood pressure control are not clear, there is significant evidence that androgens, such as *testosterone*, play an important role in gender-associated differences in blood pressure regulation (Seachrist et al., 2000; Figueroa-Valverde, 2008) . For example, there are evidences that *testosterone* is lower in populations of men with hypertension than in normal men (Fogari et al., 2001). Another case-controlled study has demonstrated an inverse relationship between testosterone and high blood pressure (Khaw and Barret, 1988).

On the other hand, some studies showed that

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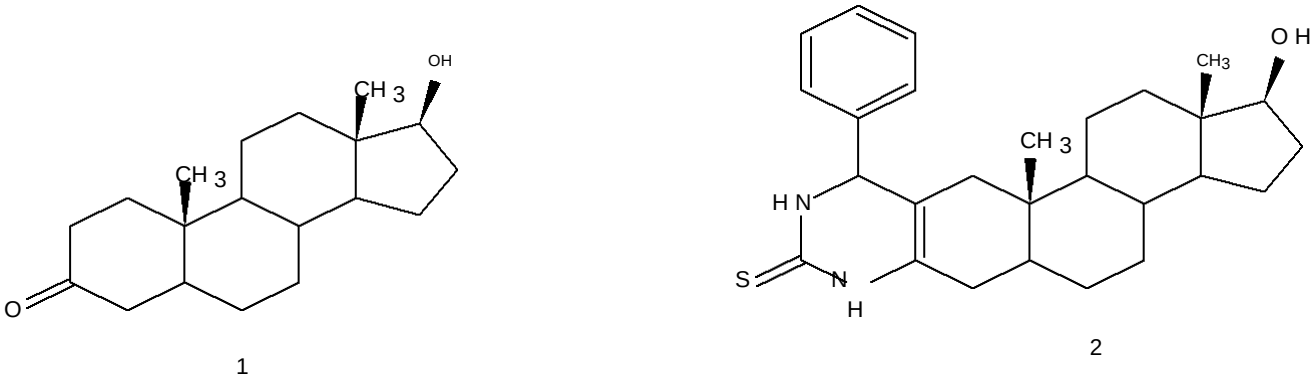

Figure 1. Chemical structure of *dihydrotestosterone* (1) and *dihydrotestosterone-dihydropyrimidine* derivative (2).

testosterone- derivatives also exert activity on blood pressure, for example, there are reports which indicate that *dihydrotestosterone* increases the blood pressure in a model of spontaneously hypertensive rats (Lengsfeld et al., 1988). Additionally, other reports suggest that effect induced by *dihydrotestosterone* on blood pressure could be via androgen-receptor (Grino et al., 1990). Nevertheless, another report (Singh et al., 2007) suggests that *dihydrotestosterone* can increase the blood pressure possibility by increasing the production of eicosanoids (20-HETE). All these data suggest that *dihydrotestosterone* exerts an effect on blood pressure. Nevertheless it is important to mention that this phenomenon could be independent of the changes in the chemical structure in A-ring of this androgen. To provide this information, the present study was designed to investigate the effects of *dihydrotestosterone* and *dihydrotestosterone-dihydropyrimidine derivative* on perfusion pressure and vascular resistance in isolated rat hearts using the Langendorff model.

Additionally, the molecular mechanism involved in the activity induced by *dihydrotestosterone- dihydropyrimidine derivative* on perfusion pressure was evaluated using several substances such as *flutamide* [antagonist of androgen receptor] (Simard et al., 1986), prazosin [α_1 adrenoreceptor antagonist] (Graham et al., 1977), *metoprolol* [selective β_1 receptor blocker] (Bengtsson et al., 1975) and *nifedipine* [inhibitor of *L- type calcium-channel*] (Henry, 1980) as pharmacological tools.

MATERIALS 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] (Jayo and Cisneros, 1996) . Male rats (Wisstar; weighing 200-250 g) were obtained from UAC.

Reagents

Dihydrotestosterone-dihydropyrimidine (Figure 1) was prepared according to a previously reported method by Figueroa (2009- a). 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 it 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. It is important to mention that perfusion medium was the Krebs -Henseleit solution (pH 7.4, 37ºC) 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 after this equilibration period.

Perfusion pressure

Evaluations of measurements of perfusion pressure changes induced by drugs application 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 *dihydrotestosterone* and *dihydrotestosterone-dihydropyrimidine derivative* on perfusion pressure

Changes in perfusion pressure as a consequence of increases in time (3-18 min) in absence (control) or presence of *dihydrotestosterone* and *dihydrotestosterone-dihydropyrimidine* 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.

Evaluation of effects exerted by *dihydrotestosterone* and *dihydrotestosterone-dihydropyrimidine derivative* on coronary resistance

The vascular resistance in absence (control) or presence of *dihydrotestosterone* and *dihydrotestosterone- dihydropyrimidine* 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).

Effects induced by *dihydrotestosterone-dihydropyrimidine* on perfusion pressure through androgen receptors

Intracoronary boluses (50 µl) of *dihydrotestosterone-dihydropyrimidine* [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).

Effect exerted by *dihydrotestosterone-dihydropyrimidine* on perfusion pressure through α_1 adrenergic receptor

Intracoronary boluses (50 µl) of *dihydrotestosterone-dihydropyrimidine* [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 *prazosin* at a concentration of 10^{-6} mM (duration of preincubation with *prazosin* was by a 10 min equilibration period).

Effects induced by *dihydrotestosterone-dihydropyrimidine* on perfusion pressure through β_1 adrenergic receptor

Intracoronary boluses (50 µl) of *dihydrotestosterone-dihydropyrimidine* [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).

Activities exerted by *dihydrotestosterone-dihydropyrimidine* on perfusion pressure through calcium-channel

Intracoronary boluses (50 µl) of *dihydrotestosterone-dihydropyrimidine* [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).

Effects induced by *caffeine* on perfusion pressure through calcium-channel

Intracoronary boluses (50 µl) of *caffeine* [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).

Statistical analysis

The obtained values are expressed as average ± SE, using each heart as its own control. The comparation between means was made with a paired Student's t test. In the case multiple comparation was used as analysis of variance (ANOVA) using the Bonferroni correction factor (Hocht et al., 1999). The differences were considered significant when *p* was equal or smaller than 0.05.

RESULTS

In this work, changes in perfusion pressure as a consequence of increases in the time (3-18 min) in absence (control) or in presence of *dihydrotestosterone* and *dihydrotestosterone-dihydropyrimidine derivative* (Figure 2) were evaluated. The results showed that *dihydrotestosterone-dihydropyrimidine derivative* [10^{-9} mM] significantly increased the perfusion pressure (***p*** = 0.005) in comparison with the control conditions and *dihydrotestosterone* (10^{-9} mM). Other results showed that vascular resistance (Figure 3), calculated as the ratio of perfusion pressure at coronary flow assayed (10 ml/min) was higher (***p*** = 0.006) in the presence of *dihydrotestosterone-dihydropyrimidine* [10^{-9} mM] than in control conditions and *dihydrotestosterone.*

Another result (Figure 4) showed that *dihydrotestosterone-dihydropyrimidine* increased the perfusion pressure in a dose dependent manner [10^{-9} to 10^{-4} mM] and this effect was not inhibited in the presence of *flutamide* [10^{-6} mM].

Data obtained of alternative experiments indicate that activity induced by *dihydrotestosterone-derivative* [10^{-9} to 10^{-4} mM] on perfusion pressure in the presence of *prazosin* (Figure 5) or *metoprolol* (Figure 6) at a concentration of 10^{-6} mM was not inhibited. Another result (Figure 7) showed that activity induced by *dihydrotestosterone-derivative* [10^{-9} to 10^{-4} mM] on perfusion pressure in the presence of *nifedipine* was significantly inhibited (p = 0.005) . Finally the effect exerted by *caffeine* [10^{-9} to 10^{-4} mM] on perfusion pressure (Figure 8) in the presence of *nifedipine* was significantly blocked (p = 0.005).

DISCUSSION

In this work, the effect induced by *dihydrotestosterone* and their derivative on the blood vessel capacity and vascular resistance translated as changes in perfusion pressure in isolated rat heart (Langendorff model) were evaluated. The results obtained showed that *dihydrotestosterone-dihydropyrimidine derivative* significantly increased the perfusion pressure through time (3-18 min) in comparison with the control conditions and *dihydrotestosterone*. Those experimental data

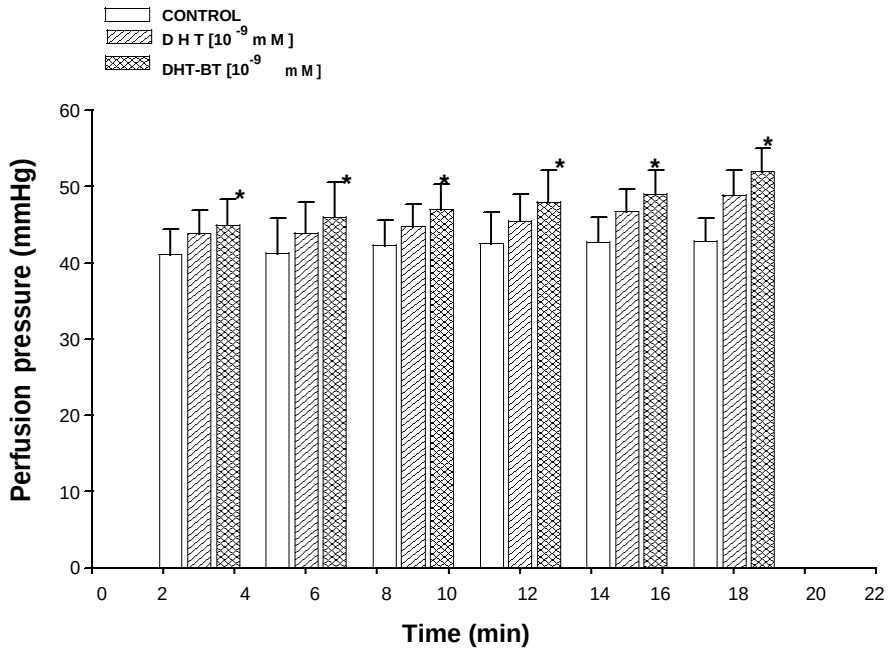

Figure 2. Effect induced by *dihydrotestosterone* (DHT) and *dihydrotestosterone-dihydropyrimidine* derivative (DHT-BT) on perfusion pressure. The results showed that *dihydrotestosterone-derivative* [10^{-9} mM] significantly increased the perfusion pressure (***p*** = 0.005) through time (3-18 min) in comparison with the control conditions and *dihydrotestosterone* [10^{-9} mM]. Each bar represents the mean ± S.E. of 9 experiments.

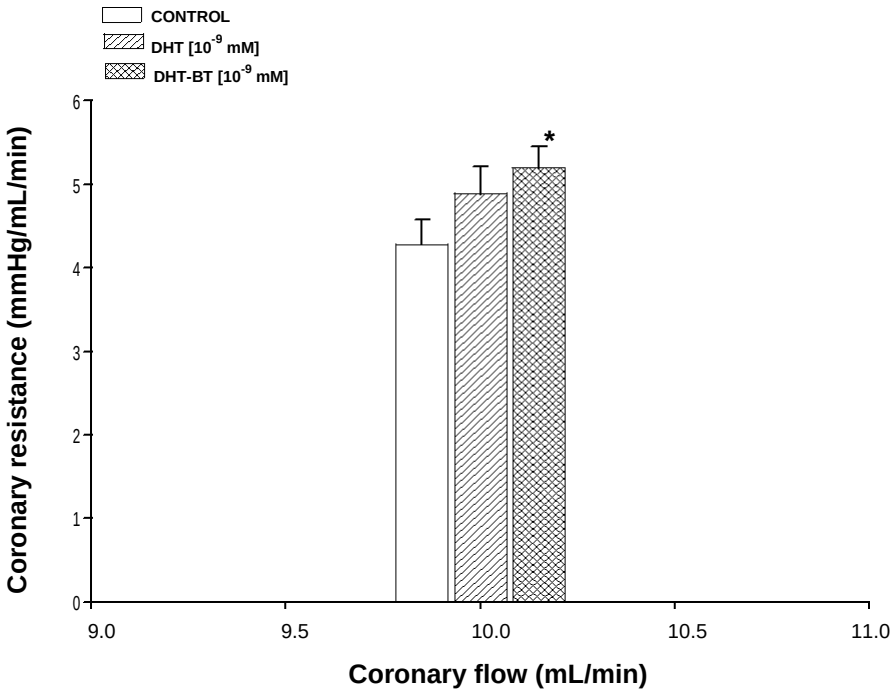

Figure 3. Activity induced by *dihydrotestosterone* (DHT) and *dihydrotestosterone-dihydropyrimidine* derivative (DHT-BT) on coronary resistance. The results showed that coronary resistance was higher (*p* = 0.006) in presence of DHT-BT [10^{-9} mM] in comparison with the control conditions and DHT [10^{-9} mM]. Each bar represents the mean ± S.E. of 9 experiments.

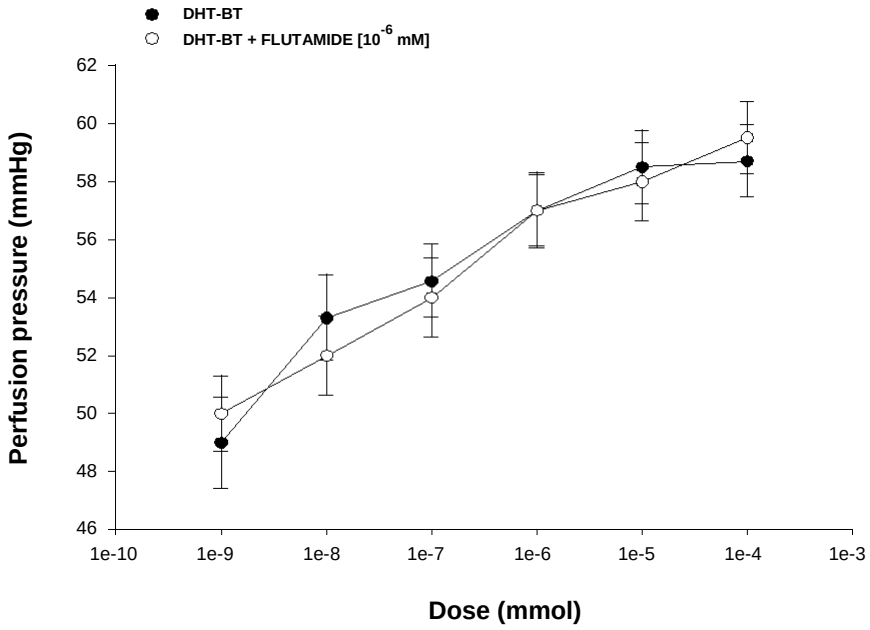

Figure 4. Effects induced by *dihydrotestosterone-dihydropyrimidine* derivative (DHT-BT) on perfusion pressure through androgen receptors. Intracoronary boluses (50 µl) of DHT-BT [10^{-9} to 10^{-4} mM]) were administered and the corresponding effect on the perfusion pressure was determined. The results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

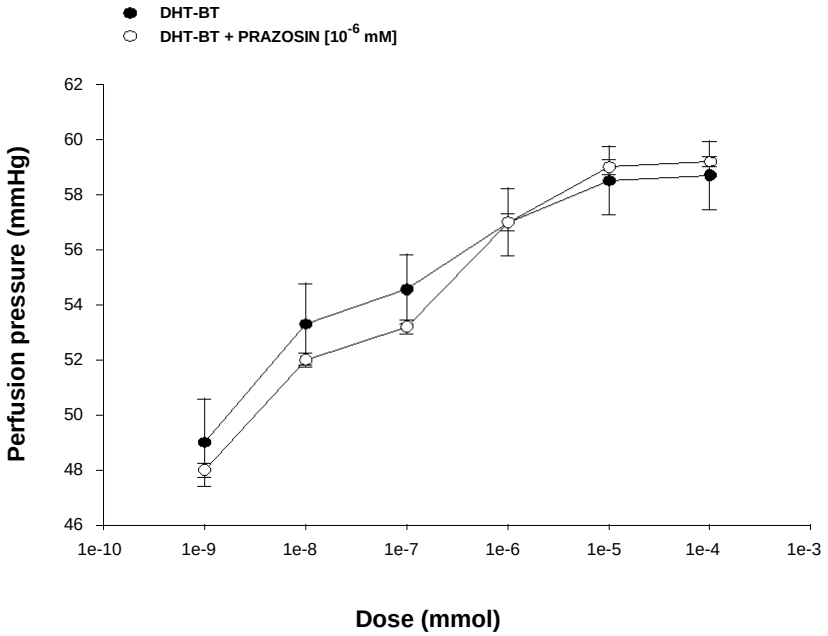

Figure 5. Effect exerted by *dihydrotestosterone-dihydropyrimidine* derivative (DHT-BT) on perfusion pressure through α_1 adrenergic receptor. DHT-BT [10^{-9} to 10^{-4} mM] was administered (intracoronary boluses, 50 µl) and the corresponding effect on the perfusion pressure was evaluated in the absence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

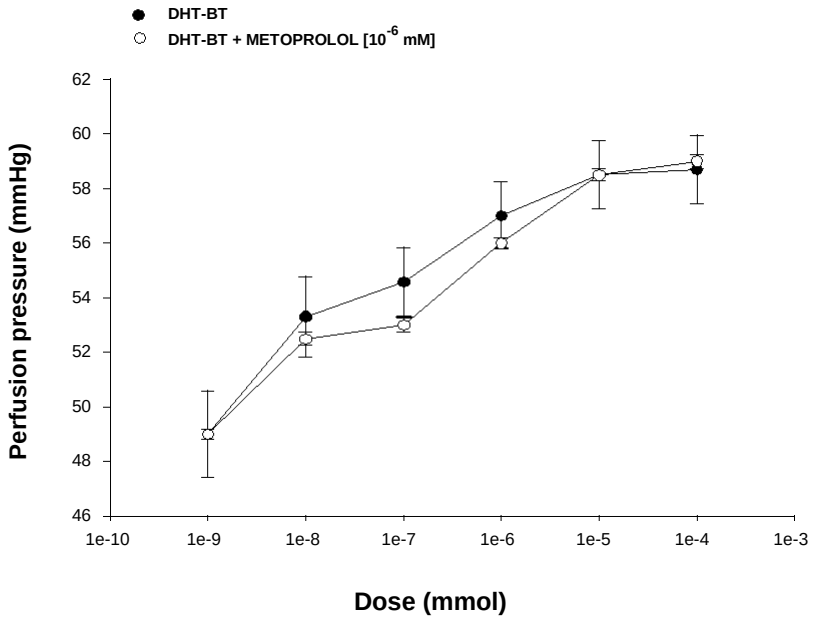

Figure 6. Activity induced by *dihydrotestosterone-dihydropyrimidine* derivative (DHT-BT) on perfusion pressure through of β_1- adrenergic receptor. Intracoronary boluses (50 µl) of DHT-BT [10^{-9} to 10^{-4} mM] were administered and the corresponding effect on the perfusion pressure was evaluated in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

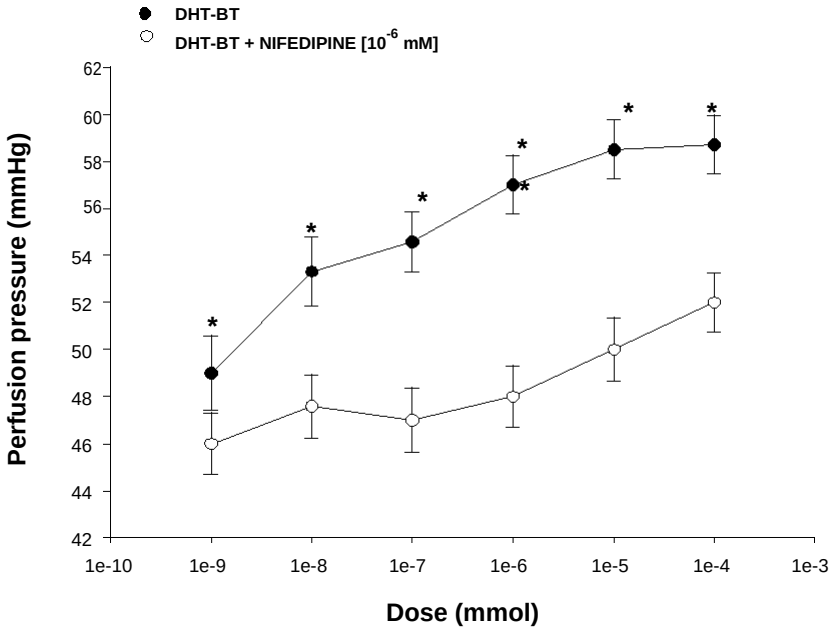

Figure 7. Effects induced by *dihydrotestosterone-dihydropyrimidine* derivative (DHT-BT) on perfusion pressure through *L- type calcium channel*. Intracoronary boluses (50 µl) of DHT-BT [10^{-9} to 10^{-4} mM] were administered in the absence and presence of *nifedipine* [10^{-6} mM]. The results showed that effect induced by DHT-BT on perfusion pressure in the presence of *nifedipine* was inhibited significantly (***p*** = 0.005). Each bar represents the mean ± SE of 9 experiments.

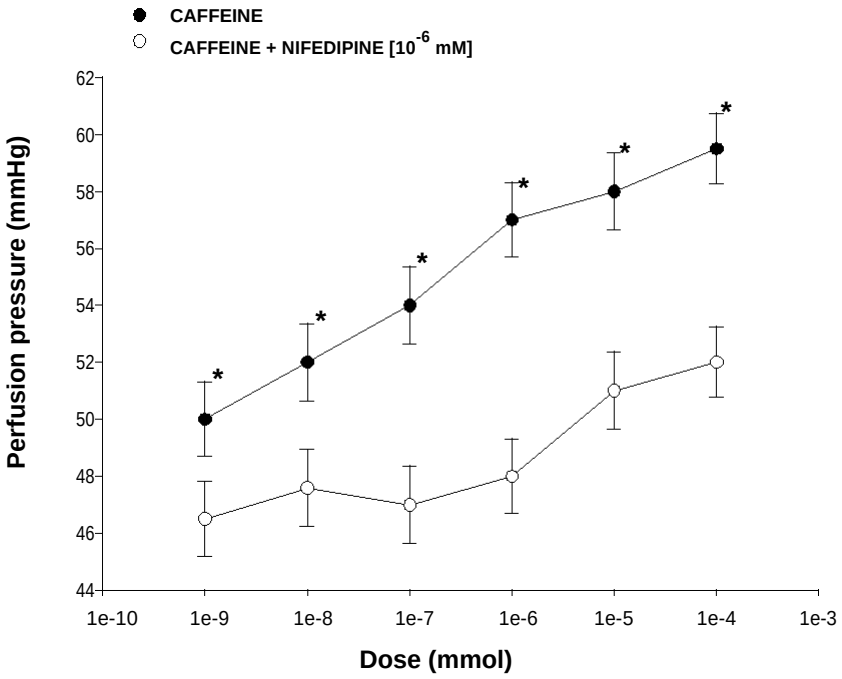

Figure 8. Activity exerted by *caffeine* on perfusion pressure through *L- type calcium channel*. Intracoronary boluses (50 µl) of *caffeine* [10^{-9} to 10^{-4} mM] were administered in absence and presence of *nifedipine* [10^{-6} mM].T he results showed that effect induced by *caffeine* on perfusion pressure in presence of *nifedipine* was inhibited significantly (p = 0.005). Each bar represents the mean ± SE of 9 experiments.

indicate that *dihydrotestosterone-dihydropyrimidine* exerts effects on perfusion pressure, which could consequently bring modifications in vascular tone and vascular resistance. In order to verify this hypothesis, the effects induced by *dihydrotestosterone* and *dihydrotestosterone-dihydropyrimidine derivative* on vascular resistance were evaluated. Our results indicate that vascular resistance in the presence of *dihydrotestosterone-dihydropyrimidine* was higher in comparison with *dihydrotestosterone* and control conditions. These experimental data suggest that changes in the chemical structure of *dihydrotestosterone* to form *dihydrotestosterone- dihydropyrimidine* induce a greater effect on the vascular tone.

On the other hand, in order to characterize the molecular mechanism of this phenomenon and analyzing the reports of Veldscholte and coworkers (1992), it is shown that *dihydrotestosterone* induces their effect via the mutated androgen receptor in some cellular lines. It is important to mention that this phenomenon could involve the interaction of *dihydrotestosterone-derivative* to androgen- receptor which may be a key requirement for activity on perfusion pressure as it happens in other types of steroids (Figueroa-Valverde et al., 2002; Figueroa, 2005). Therefore, in this study, the activity induced by *dihydrotestosterone-dihydropyrimidine* on perfusion pressure was evaluated in the presence of *flutamide* (antagonist of androgen receptor). Our results showed that effect exerted by *dihydrotestosterone-derivative* was not inhibited in the presence of *flutamide*. These experimental data suggest that molecular mechanism involved in the effect induced by *dihydrotestosterone-dihydropyrimidine* is not via androgen-receptors.

On the other hand, analyzing data obtained in this work and the molecular mechanism proposed by Kumai and coworkers (1995) suggests that some androgens can exert an indirect tonic effect on adrenal catecholamine synthesis and secretion. Additionally, other studies indicate that androgens stimulate the increased expression of *adrenergic* receptors (in some cellular lines), which have an important role in the development or maintenance of elevated blood pressure (Lilley, 1976). In order to evaluate that hypothesis in this study, the effect exerted by *dihydrotestosterone-dihydropyrimidine* on perfusion pressure was evaluated in the absence or presence of prazosin (α_1 adrenoreceptor antagonist) and metoprolol (selective β_1 receptor blocker). Our results showed that the effect induced by *dihydrotestosterone-derivative* was not inhibited in the presence of these compounds. These data indicate that molecular mechanism involved in the effects of this *androgen-derivative* on perfusion pressure is not through adrenergic activity.

Therefore, we also considered validating the effect

induced by some steroids on blood pressure via calcium channel (Figueroa, 2009-b), to evaluate the possibility that the activities exerted by *dihydrotestosterone-dihydropyrimidine* could involve increase of calcium levels. In this sense, in this study the effect induced by *dihydrotestosterone-derivative* in the absence or presence of *nifedipine* on perfusion pressure was evaluated. The results showed that activity of *dihydrotestosterone-dihydropyrimidine* in the presence of *nifedipine* was blocked significantly. These results indicate that activity exerted by steroid- derivate on perfusion pressure could involve increase of calcium through activation *L-type calcium-channel*.

In order to evaluate that premise, in this study the effect of *caffeine* on perfusion pressure was evaluated and compared with activity induced by *dihydrotestosterone-derivative*. It is important to mention that there are reports which indicate that *caffeine* indirectly increases calcium levels and blood pressure (Sitsapesan and Williams, 1990) . Our results showed that effect exerted by *caffeine* in the presence of *nifedipine* was blocked significantly in a dose-dependent manner.

These data are similar to reports of Nguyen and Myers (1988) which showed that the pressure effect of caffeine is completely reversed by subsequent nifedipine administration. Those experimental data suggest that the effect induced by caffeine on perfusion pressure was similar at the activity exerted by *dihydrotestosterone-dihydropyrimidine*. These results confirm that activity induced by *dihydrotestosterone-derivative* on perfusion pressure involves increase of calcium levels possibly through *L-type calcium-channel* activation by a non-genomic molecular mechanism.

Conclusion

The results obtained suggest that activity induced by d*ihydrotestosterone- derivative* on perfusion pressure and vascular resistance is dependent upon its chemical structure. This phenomenon possibly involves the *L-type calcium channel* activation through non-genomic molecular mechanism.

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