

*Full Length Research Paper*

# Effect induced by hemisuccinate of pregnenolone on perfusion pressure and vascular resistance in isolated rat heart

L. Figueroa-Valverde<sup>1\*</sup>, F. Díaz-Cedillo<sup>2</sup>, E. Diaz-Ku<sup>1</sup> and A. Camacho-Luis<sup>3</sup>

<sup>1</sup>Universidad Autónoma de Campeche, Av. Agustín Melgar s/n entre calle Juan de la Barrera y C-20, Col Buenavista C. P. 24039 Campeche Cam., México.

<sup>2</sup>Escuela de Ciencias Biológicas del IPN. Plan de San Luís y Díaz Mirón s/n Col. Santo Tomas, México, D. F. C.P. 11340.

<sup>3</sup>Facultad de Medicina de la Universidad Juárez del Estado de Durango, es Av. Fanny Anitua s/n Esq. Av. Universidad. C.P. 34000, Durango, Dgo., México.

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**Experimental studies suggest that pregnenolone can regulate blood pressure. Nevertheless, there is scarce information about the effects of pregnenolone and its derivatives at cardiovascular level. In addition, to date the cellular site and mechanism of action of pregnenolone at cardiovascular level is also unclear. In order, to clarify on those phenomena, we evaluated the effects of pregnenolone and hemisuccinate of pregnenolone on perfusion pressure in isolated rat heart using Langendorff flow model. Our results demonstrated that pregnenolone-derivative ( $10^{-9}$  M) significantly increase the perfusion pressure ( $p = 0.005$ ) and vascular resistance ( $p = 0.006$ ) in isolated heart. The activity exerted by hemisuccinate of pregnenolone on perfusion pressure ( $10^{-9}$  to  $10^{-4}$  M) was blocked in presence of indomethacin ( $10^{-6}$  M) and montelukast ( $10^{-6}$  M). These data suggest that activity induced by hemisuccinate of pregnenolone on perfusion pressure and vascular resistance is dependent upon its chemical structure. This phenomenon possibly involves the thromboxane  $A_2$  (TXA<sub>2</sub>) synthesis and secretion.**

**Key words:** Hemisuccinate of pregnenolone, Langendorff, perfusion pressure.

## INTRODUCTION

High blood pressure contributes substantially to cardiovascular disease incidence and premature mortality (Stary, 1989; Mahoney 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 hypertensive rat models, other investigators have found that males have higher blood pressures than do females (Ouchi et al., 1987; Ashton and Balment, 1991; Rowland and Fregly, 1992). 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). In addition, experimental and clinical studies (Khaw and Barret, 1988; Gray et al., 1991<sup>a</sup>; Gray and Feldman, 1991<sup>b</sup>) have demonstrated that steroids can be associated with hypertension development. Other studies indicate that steroids increase blood pressure affecting the renin-angiotensin system to promote the development of hypertension in male SHR (Reckelhoff et al., 1998; Grace et al., 2003).

There are other reports also showing that steroid-derivatives increase perfusion pressure and modify vascular resistance (Figueroa-Valverde et al., 2002; Figueroa et al., 2005).

Nevertheless, Li and coworkers (2004), showed that pregnenolone pretreatment prevents elevation of tall artery systolic pressure in rats using a stress-induced hypertension model and that effect induced by pregnenolone involve reduction of angiotensin II levels. More so, other reports indicate that pregnenolone derivative (allo-

\*Corresponding author. E-mail: lauro\_1999@yahoo.com. Tel.: (981) 8119800 Ext. 73006. Fax (981) 8119800 Ext. 73002.

pregnenolone) can regulate blood pressure in hypertensive patients (Luisi et al., 2000), possibility by stimulating catecholamine synthesis and secretion (Charalampopoulos et al., 2005). Other studies showed that 20R 14 $\beta$ -amino-3 $\beta$ -rhamnosyl-5 $\beta$ -pregnan-20 $\beta$ -ol can also induce a positive inotropic action in a dog model of induced heart failure. This effect by this steroid-derivative could be through its high affinity to Na<sup>+</sup>, K (+)-ATPase receptor (Maixen et al., 1992). Apart from the above experiments, which also don't show clearly the cellular site and actual molecular mechanisms of pregnenolone, data information is needed for characterizing the activity induced by this steroid at cardiovascular level. To provide this information, the present study was designed to investigate the effects of pregnenolone and hemisuccinate of pregnenolone on perfusion pressure and vascular resistance in isolated rat hearts using Langendorff model. In addition, the molecular mechanism involved in the activity induced by pregnenolone-derivative on perfusion pressure was evaluated using several substances such as flutamide (antagonist of androgen receptor) (Simard et al., 1986) prazosin ( $\alpha_1$  adrenoreceptor antagonist) (Graham et al., 1977), metoprolol (selective  $\beta_1$  receptor blocker) (Bengtsson et al., 1975), indomethacin (inhibitor of prostaglandin synthesis) (Owen et al., 1975) and montelukast (antagonist of thromboxane A<sub>2</sub>) (Okamoto et al., 2006) were used 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). Male rats (Wisstar; weighing 200-250g) were obtained from UAC.

### Reagents

Pregnen-20-one-3-(3-carboxy-1-oxopropoxy) (hemisuccinate of pregnenolone) was prepared according to a previously reported method by Figueroa et al., (2006). 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 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. It is important to mention that perfusion medium was the Krebs-Henseleit solution (pH 7.4, 37°C) composed of (mM); 117.8 NaCl; 6 KCl; 1.75 CaCl<sub>2</sub>; 1.2 NaH<sub>2</sub>PO<sub>4</sub>; 1.2 MgSO<sub>4</sub>; 24.2 NaHCO<sub>3</sub>; 5 glucose, and 5 sodium pyruvate. The solution was actively bubbled with a mixture of O<sub>2</sub>/CO<sub>2</sub> (95:5). The co-

ronary 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 pregnenolone and hemisuccinate of pregnenolone on perfusion pressure Changes in perfusion pressure as a consequence of increases in time (3-18 min) in absence (control) or presence of pregnenolone and hemisuccinate of pregnenolone at a concentration of 10<sup>-9</sup> M were determined. The effects were obtained in isolated hearts perfused at a constant-flow rate of 10 ml/min (Ceballos et al., 1999)

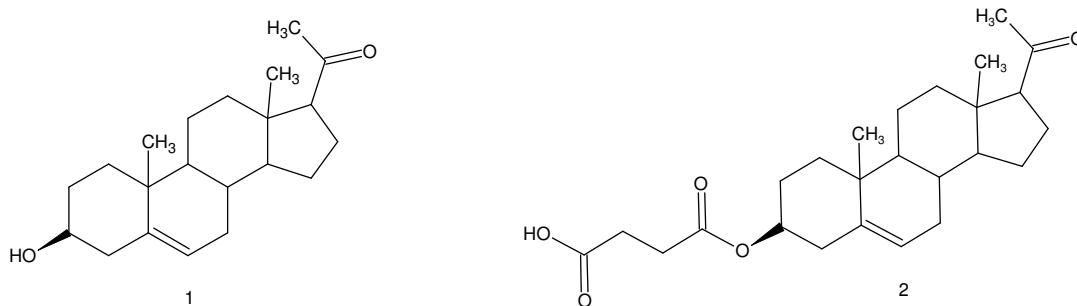
Evaluation of effects exerted by pregnenolone and hemisuccinate-pregnenolone on vascular resistance. The vascular resistance in absence (control) or presence of pregnenolone and hemisuccinate of pregnenolone at a concentration of 10<sup>-9</sup> M was evaluated. The effects were obtained in isolated hearts perfused at a constant-flow rate of 10 ml/min. The vascular resistance was determined by the relationship between coronary flow and perfusion pressure (mm Hg/ml/min) (Figueroa et al., 2005).

Effects induced by hemisuccinate-pregnenolone on perfusion pressure through androgen receptors Intracoronary boluses (50  $\mu$ l) of hemisuccinate of pregnenolone (10<sup>-9</sup> to 10<sup>-4</sup> M) 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<sup>-6</sup> M (duration of preincubation with flutamide was by a 10 min equilibration period) (Ceballos et al., 1999).

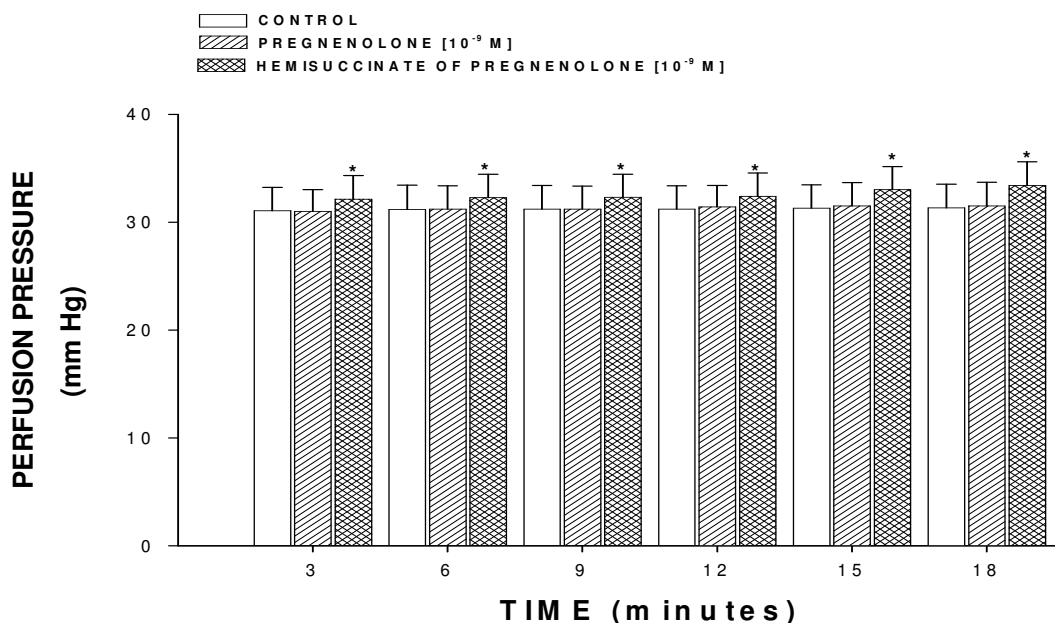
Effect exerted by hemisuccinate-pregnenolone on perfusion pressure through of  $\alpha_1$  adrenergic receptor Intracoronary boluses (50  $\mu$ l) of hemisuccinate of pregnenolone (10<sup>-9</sup> to 10<sup>-4</sup> M) were administered and the corresponding effect on the perfusion pressure was evaluated. The dose-response curve (control) was repeated in the presence of prazosin at a concentration of 10<sup>-6</sup> M (duration of preincubation with prazosin was by a 10 min equilibration period) (Drew et al., 1979).

Effects induced by hemisuccinate-pregnenolone on perfusion pressure through of  $\beta_1$  adrenergic receptor Intracoronary boluses (50  $\mu$ l) of hemisuccinate of pregnenolone (10<sup>-9</sup> to 10<sup>-4</sup> M) 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<sup>-6</sup> M (duration of preincubation with metoprolol was by a 10 min equilibration period) (Rajala et al., 1988)

Activities exerted by hemisuccinate-pregnenolone on perfusion pressure through of synthesis of prostaglandins intracoronary boluses (50  $\mu$ l) of hemisuccinate of pregnenolone (10<sup>-9</sup> to 10<sup>-4</sup> M) were administered and the corresponding effect on the perfusion pressure was evaluated. The dose-response curve (control) was repeated in the presence of indomethacin at a concentration of 10<sup>-6</sup> M (duration of preincubation with indomethacin was by a 10 min equilibration period) (Figueroa et al., 2005). Effects induced by hemisuccinate-pregnenolone on perfusion pressure through of thromboxane A<sub>2</sub> synthesis Intracoronary boluses (50  $\mu$ l) of hemisuccinate of pregnenolone (10<sup>-9</sup> - 10<sup>-4</sup> M) were administered and the corresponding effect of the perfusion pressure was evaluated. The dose-



**Figure 1.** Chemical structure of pregnenolone (1) and hemisuccinate of pregnenolone (2).



**Figure 2.** Effect induced by pregnenolone and hemisuccinate of pregnenolone on perfusion pressure. The results showed that pregnenolone-derivative ( $10^{-9}$  M) significantly increase the perfusion pressure ( $p = 0.005$ ) through of time (3-18 minutes) in comparison with the control conditions and pregnenolone ( $10^{-9}$  M). The effect is expressed as the area under the curve, and each bar represents the mean  $\pm$  S.E. of 9 experiments

response curve (control) was repeated in the presence of montelukast at a concentration of  $10^{-6}$  M (duration of preincubation with montelukast was by a 10 min equilibration period). It is important to mention that the concentration used of montelukast in this study was product of experimental data found (not showed) which involve dose-effect curve.

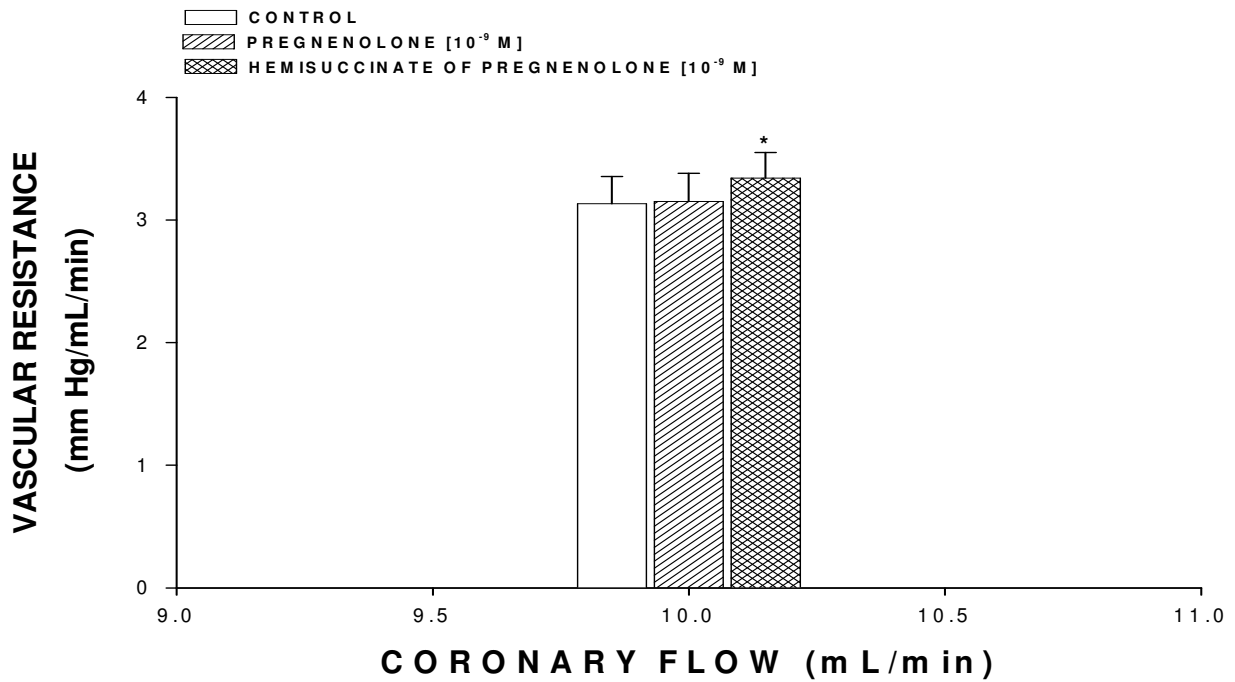
#### Statistical analysis

The obtained values are expressed as average  $\pm$  SE, using each heart as its own control. The data obtained were put under an 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 0.01.

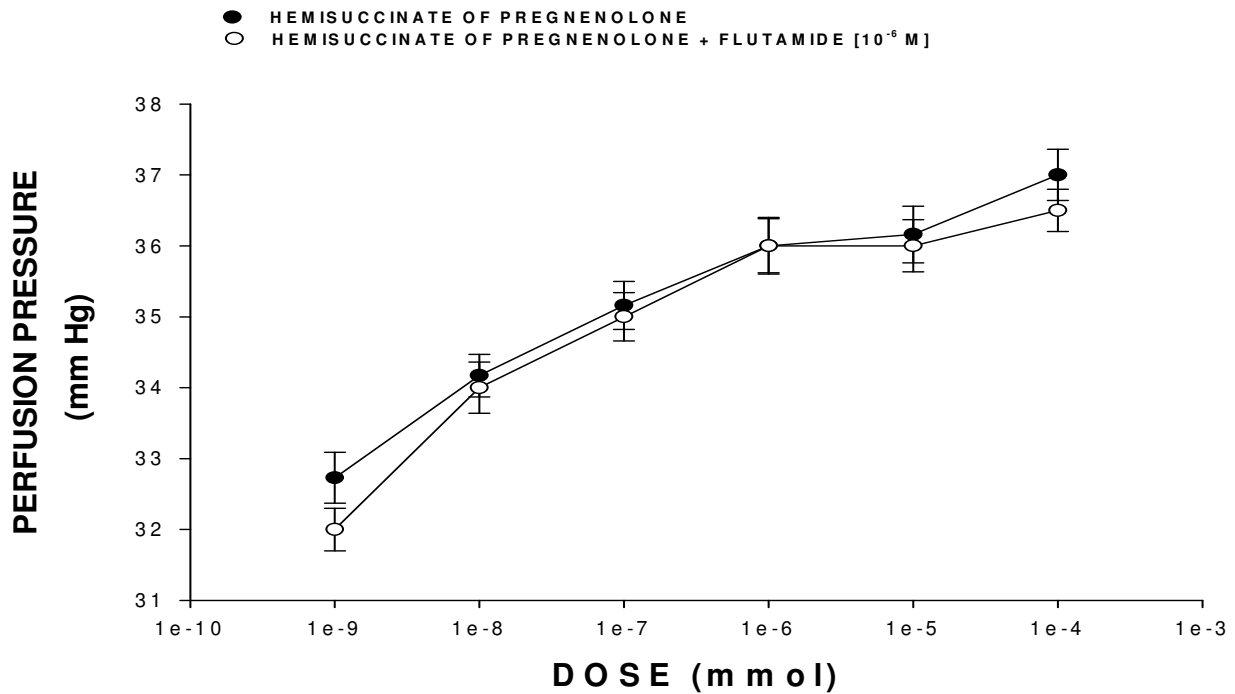
#### RESULTS

The results obtained from changes in perfusion pressure as a consequence of increases in the time (3-18 min) in

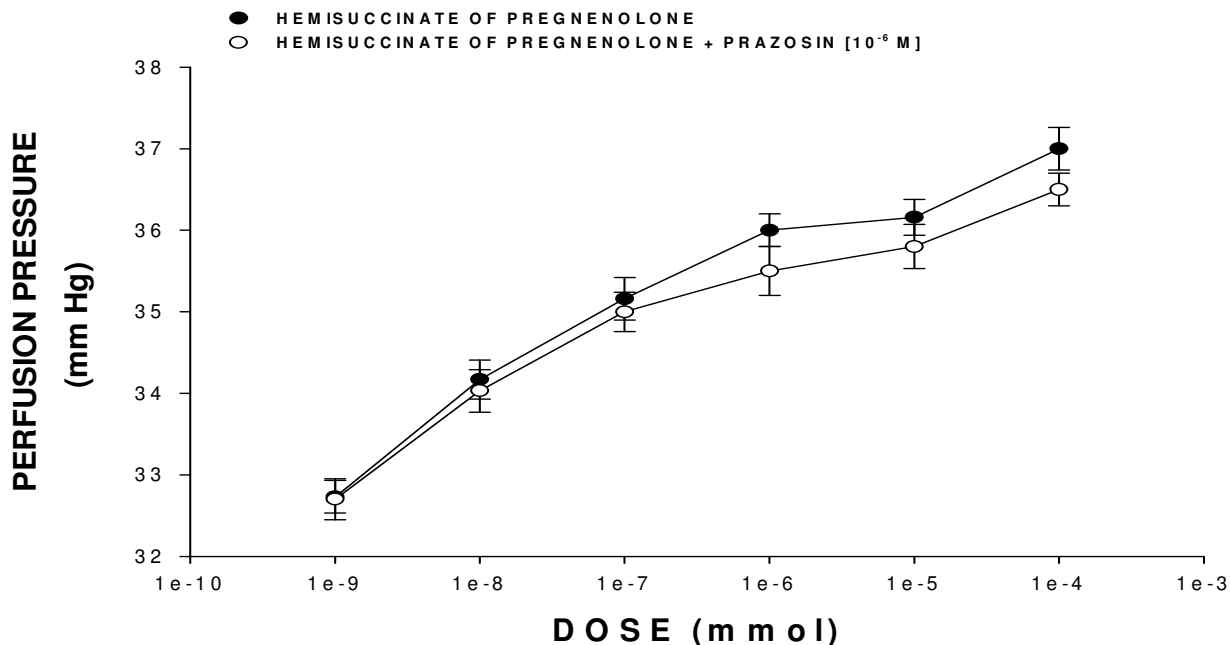
absence (control) or in presence of pregnenolone and hemisuccinate-pregnenolone (Figure 2), showed that pregnenolone-derivative ( $10^{-9}$  M) significantly increase the perfusion pressure ( $p = 0.005$ ) in comparison with the control conditions and pregnenolone ( $10^{-9}$  M). Another result showed that vascular resistance, calculated as the ratio of perfusion pressure at coronary flow assayed (10 ml/min) was higher ( $p = 0.006$ ) in the presence of hemisuccinate-pregnenolone ( $10^{-9}$  M) than in control conditions and pregnenolone (Figure 3). Other experiments (Figure 4) showed that hemisuccinate-pregnenolone increase the perfusion pressure in a dose dependent manner ( $10^{-9}$  to  $10^{-4}$  M) and this effect was not inhibited in presence of flutamide ( $10^{-6}$  M). Alternative experimental showed that activity induced by pregnenolone-derivative ( $10^{-9}$  to  $10^{-4}$  M) on perfusion pressure in presence of prazosin (Figure 5) or metoprolol (Figure 6) at a concen-



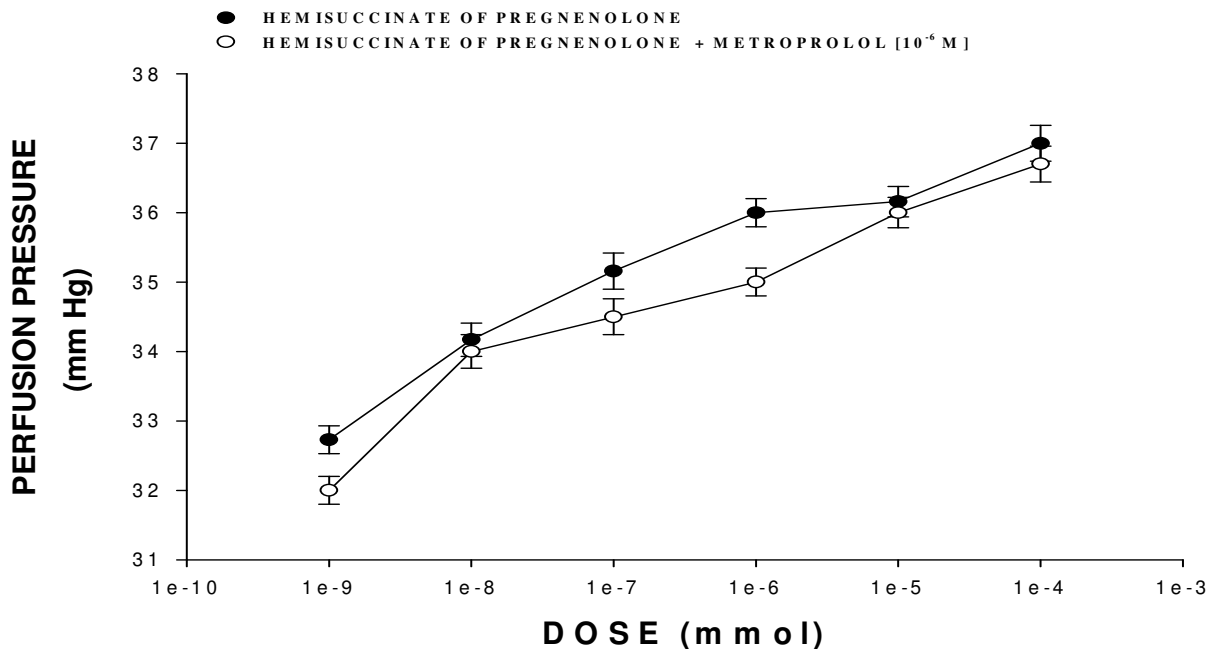
**Figure 3.** Activity induced by pregnenolone and hemisuccinate-pregnenolone on vascular resistance. The results showed that vascular resistance was higher ( $p = 0.006$ ) in presence of hemisuccinate-pregnenolone ( $10^{-9}$  M) in comparison with the control conditions and pregnenolone ( $10^{-9}$  M). The effect it is expressed as the area under the curve, and each bar represents the mean  $\pm$  S.E. of 9 experiments.



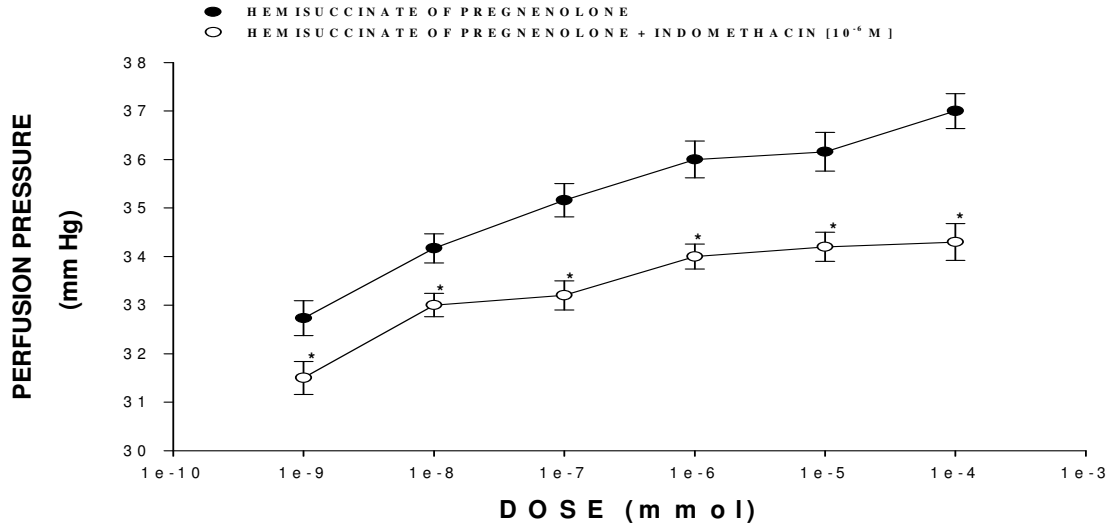
**Figure 4.** Effects induced by hemisuccinate-pregnenolone on perfusion pressure through androgen receptors. Intracoronary boluses (50  $\mu$ l) of hemisuccinate of pregnenolone ( $10^{-9}$  to  $10^{-4}$  M) were administered and the corresponding effect on the perfusion pressure was determined. The results showed that hemisuccinate-pregnenolone increase the perfusion pressure in a dependent dose manner and this effect was not inhibited in presence of flutamide ( $10^{-6}$  M). The effect it is expressed as the area under the curve, and each bar represents the mean  $\pm$  S.E. of 9 experiments.



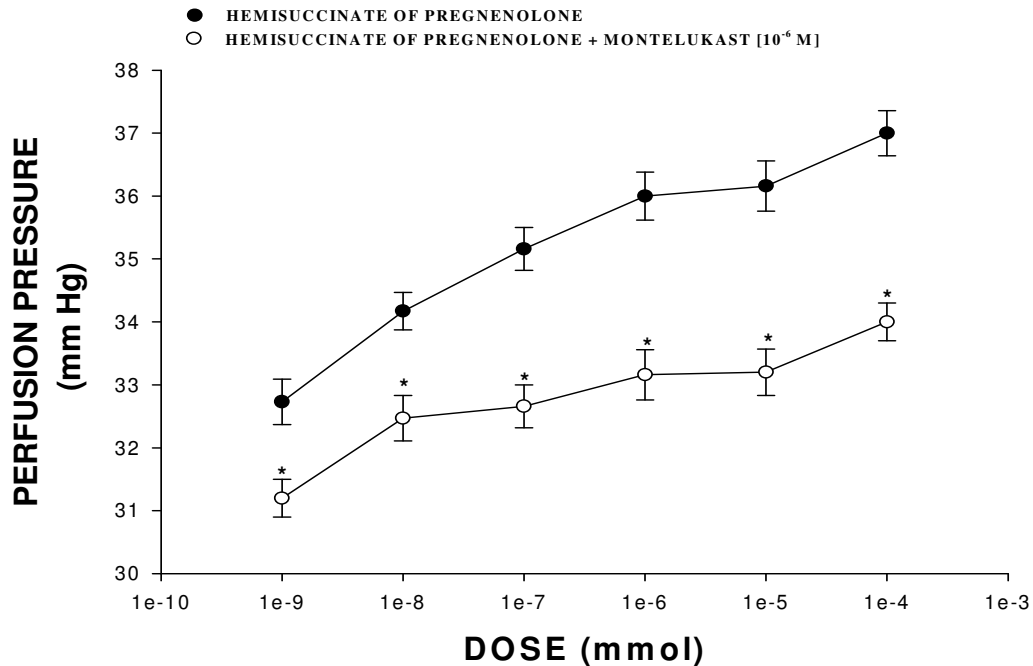
**Figure 5.** Effect exerted by hemisuccinate-pregnenolone on perfusion pressure through of  $\alpha_1$  adrenergic re-ceptor. Hemisuccinate of pregnenolone ( $10^{-9}$  to  $10^{-4}$  M) was administered (intracoronary boluses, 50  $\mu$ l) and the corresponding effect on the perfusion pressure was evaluated in absence and presence of prazosin ( $10^{-6}$  M). The results showed that activity induced by pregnenolone-derivative on perfusion pressure was not inhi-bited in presence of prazosin. The effect it is expressed as the area under the curve, and each bar represents the mean  $\pm$  S.E. of 9 experiments.



**Figure 6.** Activity induced by hemisuccinate-pregnenolone on perfusion pressure through of  $\beta_1$ - adrenergic receptor. Intracoronary boluses (50  $\mu$ l) of hemisuccinate of pregnenolone ( $10^{-9}$  to  $10^{-4}$  M) were administered and the corresponding effect on the perfusion pressure was evaluated in absence and presence of metoprolol ( $10^{-6}$  M). The results showed that activity induced by pregnenolone-derivative on perfusion pressure was not inhibited in presence of metoprolol. The effects it is expressed as the area under the curve, and each bar represents the mean  $\pm$  S.E. of 9 experiments.



**Figure 7.** Effects exerted by hemisuccinate-pregnenolone on perfusion pressure through of synthesis of prostaglandins. Pregnenolone-derivative ( $10^{-9}$  to  $10^{-4}$  M) was administered (intracoronary boluses, 50  $\mu$ l) and the corresponding effect on the perfusion pressure was evaluated in absence and presence and indometacin ( $10^{-6}$  M). The results showed that activity induced by hemisuccinate-pregnenolone on perfusion pressure in presence of indomethacin was inhibited significantly ( $p = 0.005$ ). The effect it is expressed as the area under the curve, and each bar represents the mean  $\pm$  S.E. of 9 experiments.



**Figure 8.** Effects induced by hemisuccinate-pregnenolone on perfusion pressure through of thromboxane  $A_2$  synthesis. Intracoronary boluses (50  $\mu$ l) of hemisuccinate of pregnenolone [ $10^{-9}$  to  $10^{-4}$  M] were administered in absence and presence of montelukast ( $10^{-6}$  M).The results showed that effect induced by pregnenolone-derivative on perfusion pressure in presence of montelukast was inhibited significantly ( $p = 0.005$ ). The effect it is expressed as the area under the curve, and each bar represents the mean  $\pm$  SE of 9 experiments.

tration of  $10^{-6}$  M was not inhibited. Finally the activity exerted by hemisuccinate-pregnenolone ( $10^{-9}$  to  $10^{-4}$  M) on perfusion pressure (Figure 7) in presence of indome-

thacin ( $10^{-6}$  M) was inhibited significantly ( $p = 0.005$ ). Additionally, the effect induced by pregnenolone-derivative ( $10^{-9}$  to  $10^{-4}$  M) on perfusion pressure (Figure 8)

in presence of montelukast ( $10^{-6}$  M) was inhibited significantly ( $p = 0.005$ ).

## DISCUSSION

In this study, the effect induced by pregnenolone and their derivative on the blood vessel capacity and vascular resistance translated as changes in perfusion pressure in isolated rat heart (Langendorff model) was evaluated. The results obtained (Figure 2) indicating that hemisuccinate-pregnenolone ( $10^{-9}$  M) significantly increased the perfusion pressure ( $p = 0.005$ ) through of time (3-18 min) in comparison with the control conditions and pregnenolone ( $10^{-9}$  M). Those experimental data indicate that pregnenolone-hemisuccinate 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 pregnenolone and pregnenolone-derivative on vascular resistance were evaluated. The results indicate that vascular resistance (Figure 3) in presence of pregnenolone-hemisuccinate was higher in comparison with pregnenolone and control conditions. Therefore, these experimental data suggest that changes in the chemical structure of pregnenolone to form hemisuccinate of pregnenolone (Figure 1) induce a greater effect on the vascular tone.

In order to characterize the molecular mechanism of this phenomenon and analyze the reports of Grigoryev et al. (2000), which showed that pregnenolone induce their effect via the mutated androgen receptor in some cellular lines. It is important to mention that this phenomenon could involve the interaction of steroid-derivative to androgen-receptor which may be key requirement for activity as happens in other types of steroids (Figuroa-Valverde et al., 2002).

Therefore, in this study, the activity induced by hemisuccinate of pregnenolone on perfusion pressure was evaluated in presence of flutamide (antagonist of androgen receptor). The results showed that effect exerted by pregnenolone-derivative ( $10^{-9}$  to  $10^{-4}$  M) was not inhibited in presence of flutamide ( $10^{-6}$  M). These experimental data indicate that molecular mechanism involved in the effect induced by hemisuccinate of pregnenolone is not via androgen-receptors. On the other hand, analyzing data obtained and the molecular mechanism proposed by Charalampopoulos et al. (2005) which suggests that allopregnenolone exert a direct tonic effect on adrenal catecholamine synthesis and secretion. Additionally, other studies showed that pregnenolone-derivative (allopregnenolone) stimulate adrenergic activity, which has an important role in the development or maintenance of elevated blood pressure (Lilley et al., 1976). In order, to evaluate this hypothesis in this study, the effect exerted by hemisuccinate of pregnenolone on perfusion pressure was evaluated in absence or presence of prazosin ( $\alpha_1$  adrenoreceptor antagonist) and metoprolol (selective  $\beta_1$  receptor blocker). The results showed that effect induced

by pregnenolone-derivative was not inhibited in presence of these compounds. These data indicate that molecular mechanism involved in the effects of this steroid-derivative on perfusion pressure is not through adrenergic activity. We also considered validating the effect induced by some steroids on perfusion pressure via-prostaglandins (Sheillan et al., 1983) and to evaluate the possibility that the activities exerted by hemisuccinate of pregnenolone involve stimulation and secretion of prostaglandins. In this study we evaluated the effect exerted by pre-gnenolone-derivative in absence or presence of indomethacin ( $10^{-6}$  M). The results showed that activity of hemisuccinate of pregnenolone in presence of indomethacin was blocked significantly ( $p = 0.005$ ). These results indicate that activity exerted by steroid-derivate on perfusion pressure involve the prostanoids synthesis and secretion. Analysis on the possibility of hemisuccinate of pregnenolone inducing their activity on perfusion pressure through syntheses of thromboxane  $A_2$  (TXA<sub>2</sub>) as it happens in another type of steroids (Schork et al., 1994) was done. In this study the effect of pregnenolone-derivative in absence and presence of montelukast (antagonist of non-specific of synthesis of TXA<sub>2</sub>) on perfusion pressure was evaluated. The experimental data showed that effect of pregnenolone-derivative on perfusion pressure in presence of montelukast was inhibited significantly ( $p = 0.005$ ). Nevertheless, it is important to mention that activity induced by hemisuccinate of pregnenolone in presence of montelukast (Figure 8) is high in comparison with the effect exerted by indomethacin (Figure 7). These results suggest that activity of pregnenolone-derivative could be indirect through other additional molecular mechanisms, which could possibly involve other vasoconstrictor-substances.

## Conclusions

Our data suggest that activity induced by hemisuccinate of pregnenolone on perfusion pressure and vascular resistance depends on chemical structure. This phenomenon possibly involves thromboxane  $A_2$  (TXA<sub>2</sub>) synthesis and secretion.

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