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RESEARCH ARTICLE

Effects of Indomethacin on the Norepinephrine-Induced Contraction in Vascular Smooth Muscle during Different Stages of the Estrous Cycle

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"Dedicated to Dr. Berta Zamorano"

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A B S T R A C T

Previous studies have shown that vascular reactivity evaluated in female rat aorta to norepinephrine did not present the same response at all periods of their estrous cycle. During proestrus, the contractile response shows lower values. It was also reported that vascular reactivity had a close relationship with prostaglandins activity. In this study, we studied the response to norepinephrine in rat aorta rings treated with indomethacin. Indomethacin is a well-known inhibitor of prostaglandin synthesis. At low concentrations, the effect of indomethacin was not significant, and vascular reactivity response to norepinephrine was similar to those aortic rings from the control rats (without indomethacin). However, at high concentrations of norepinephrine, a marked difference was observed. The inhibitory action of indomethacin on the synthesis of prostaglandins was effectively reflected in a lower release of prostaglandins. In this way, our results confirm previous findings and indicate that indomethacin increases the constrictor response to norepinephrine, avoiding the vasodilatory effect of prostaglandins.

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INTRODUCTION

The hormonal differences in different genders influence the development of cardiovascular diseases both in humans and animals (Dantas *et al.,* 2014). Sex can determine differences in the regulation of vascular reactivity which, in turn, can explain differences in the incidence of cardiovascular diseases like hypertension, stroke, and atherosclerosis (Lawler *et al.*, 1995; Jamieson and Skliut, 2010). This difference hormonal profile due to difference in gender can also lead to misinterpretation of experimental results if they are not taken into account. This effect is evident from several studies, which have shown that the estrous cycle influences the response to agonist. Induction of contracted rat aorta by norepinephrine (Zamorano *et al*., 1994), reactivity in the mesenteric vascular bed of rat (Dalle Lucca *et al.*, 2000) and the reactivity of sheep uterine arteries (Sprague *et al*., 2009) are few examples of it. In the same way, the relationship established with the PGs shows that both prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) dilate the vascular smooth muscle (Sprague *et al*., 2009).

Indomethacin has been widely used as a pharmacological tool (Shirota *et al.*, 1998; Poptrajkovic *et al.*, 2002). This compound significantly decreases the average level of serum progesterone, regardless of whether the pregnancy is allogeneic or syngeneic (Poptrajkovic *et al.*, 2002). It has been shown that indomethacin reduced the vascular response to α-adrenoceptor agonists in the endothelium-denuded aorta of rat, which could be due to the prevention of vasoconstrictor PGs formation in smooth muscles of aorta by COX-2. (Lopez *et al.*, 2017). Since indomethacin inhibits the release of PGs, it is possible to speculate that the availability of PGs may influence the contractile responses (Jaimes *et al*., 2019). Thus, present study was planned to study the relationship that would exist between the vascular contractile responses of the vascular smooth

muscle of rat using aortic rings to norepinephrine in the presence of indomethacin.

MATERIALS AND METHODS

Animals: A total of 15 female Sprague-Dawley rat strains were maintained and housed in groups of 4 rats per cage with 12:12 h dark/light cycle along with the free access to water and food. Initial body weight was 220±5 g (mean ± SD), and the mean blood pressure was 104±3 Torr. Vaginal smears were taken daily to determine different stages of estrous cycle as descript by (Vilela *et al.*, 2007).

Vascular reactivity: The excess connective tissues and fat were removed from the isolated thoracic aorta. The vessels were mounted in organs baths by cutting them into a length of 5 mm rings, according to the technique described by Illanes *et al.* (1993). Briefly, fat and connective tissues were cleaned by careful removal of thoracic aortas. A 30 mL bath of modified Krebs-Henseleit solution on a stainless steel hook was used to mount the aortic rings with resting tension of 1.5 g. The maximum contraction force of the ring was obtained by contracting the rings three times at least and their tension was estimated to be 100% by the depolarizing high K^+ Krebs-Henseleit solution. This further reduced NaCl to 56.7 mM and increased KCl by 70 mM. Maximum tension was developed in aortic rings by depolarizing solution. Endothelium integrity was evaluated by testing the resulting relaxation by acetylcholine (1 µM) addition in rings precontracted by norepinephrine (0.1 µM). Baseline relaxation was considered to be 100% and thus, response was evaluated by measuring percentage relaxation from the precontracted level of relaxation (Illanes *et al.*, 1993). All the experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (1985), NIH, Bethesda, USA 2011 and the Committee of Bioethics of the University of Santiago of Chile.

Measurement of cyclooxygenase: Prostaglandins (PGE_2 and $PGF_{2\alpha}$) measurements were done according to the procedure described by Zamorano *et al.* (1995). The method used for the determination of prostanoids was performed through the radioimmunoassay assay described by Zamorano *et al.* (1991; 1995). Proteins quantification were carried out according to Lowry *et al.* (1951).

Statistical analysis: Data were expressed as means ± SEM after obtaining from vascular reactivity studies. Statistical significance of results of vascular reactivity was evaluated by analysis of variance (ANOVA) followed by Tukey test. A computer program GB-STAT 3.0 was used to perform all these analyses. Significant differences were considered if the obtained probability values were less than 0.05.

RESULTS

Vascular reactivity: Concentration-response curve of the tension induced by NE in the presence of indomethacin in aortic rings in proestrous stage obtained from rats has been shown in Fig 1. Figure 1-4 show the cumulative dose-response curves of NE-induced contraction of aortic rings excised from rats at the 4 different stages of the estrous cycle. These curves were virtually identical for aortic rings, tested with NE in a dose range to 10^{-12} to 10^{-6} M.

In the same way, Table 1 shows the maximum effect and pD_2 in the absence and presence of indomethacin, with two important issues being observed, the increase in the maximum effect coinciding with the response observed in Figures 1 to 4, but a non-significant decrease of the pD_2 in those experiments that were in the presence of indomethacin compared to those not inducing indomethacin.

Figure 5 shows the effects of indomethacin on cyclooxygenase release. The effect of cyclooxygenase release was observed in control rats and in rats treated with indomethacin. It is observed that in estrous, metestrus and diestrus the results are similar while there are significant differences in proestrus.

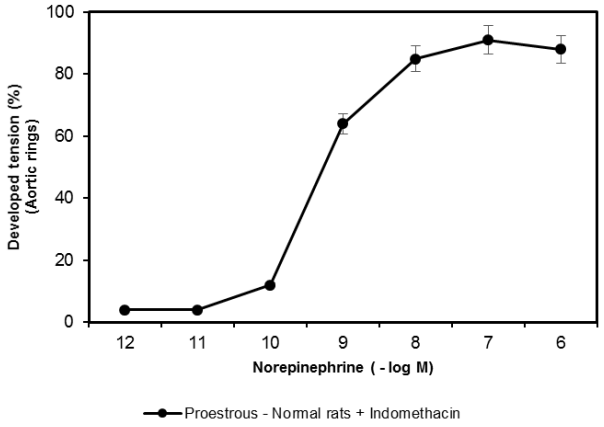

Fig. 1: Effects of indomethacin on the NE-induced aortic ring contraction in normal rats in proestrus.

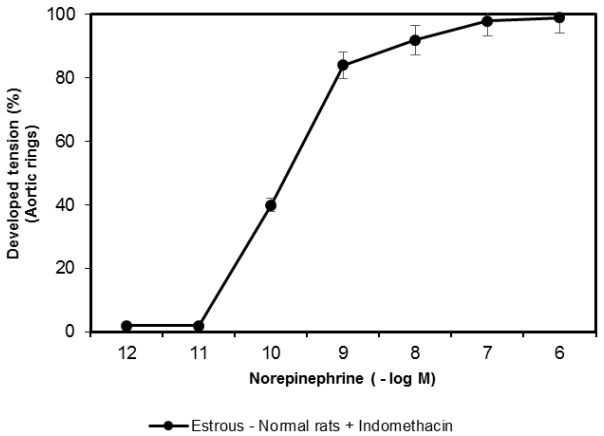

Fig. 2: Effects of indomethacin on the NE-induced aortic ring contraction in normal rats in estrous.

Table 1: Effect of indomethacin on norepinephrine-induced contraction in rat aorta at different stage of estrous cycle

	E_{max} + Ind	pD_2 + Ind
Proestrous	84.20±3.00	9.30±0.09
Estrous	99.61±2.85	9.52±0.07
Metaestrous	90.00±3.26	9.50±0.11
Diestrous	99.26±1.89	9.81±0.06

E_{max}=(maximal contraction by agonist / maximal contraction by 70 mM KCl) x 100; pD_2=-log ED_{50}; Ind=indomethacin; n=12 rats in each stage of estrous cycle without indomethacin and 6 with indomethacin. *=P<0.001 between proestrous with other periods of estral cycle.

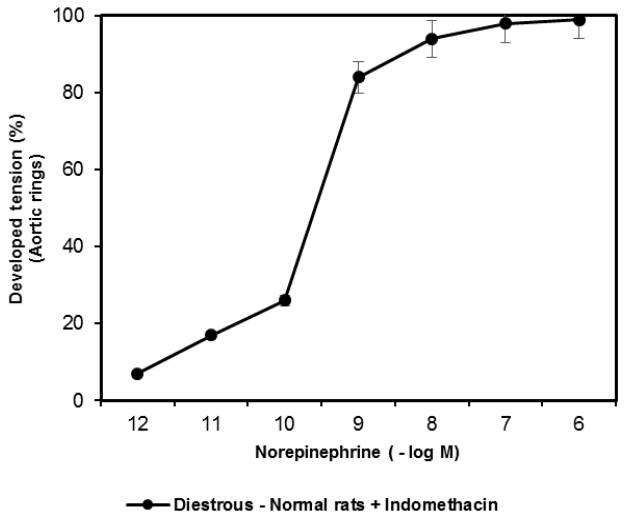

Fig. 3: Effects of indomethacin on the NE-induced aortic ring contraction in normal rats in diestrous.

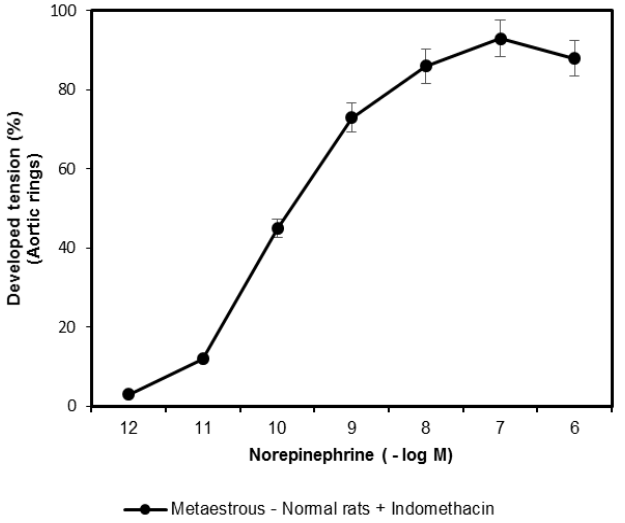

Fig. 4: Effects of indomethacin on the NE-induced aortic ring contraction in normal rats in metaestrous.

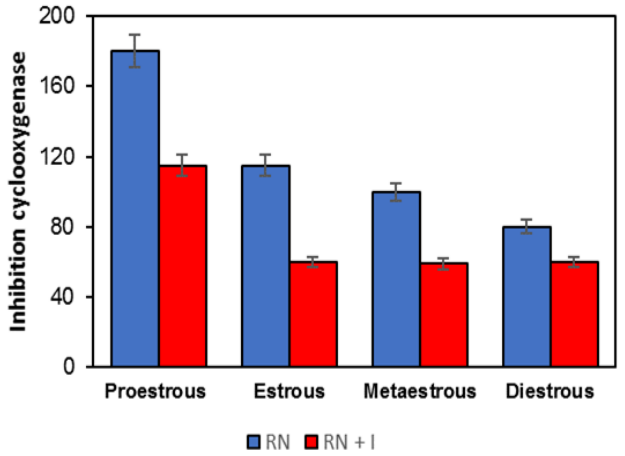

Fig. 5: Effect of indomethacin on cyclooxygenase release. Blue bar: Normal rat aorta controls; Red bar: Normal rat aorta + indomethacin; n=20 in each case; the graph shows mean±standard error in pg/mg wet tissue/min. A significant difference is observed when comparing control rats with indomethacin-treated rats in each stage of the estral cycle. In proestrous, estrous and metaestrous (*) present P<0.001 between without and with indomethacin; In diestrous (**) P<0.005. Figure 5 shows the effect of indomethacin on prostaglandin-E_2 (PGE_2) concentration in rat aorta at different estral stages.

DISCUSSION

The estrous cycle of the rat has been well characterized (Vilela *et al.*, 2007). On the other hand, the norepinephrine constrictor response in the rat aorta, as well as its relationship with vascular biosynthesis of prostanoids, has also been studied (Zamorano *et al.*, 1994). The effect of prostaglandins $PGF_{2\alpha}$ and PGE_2 has also been investigated in rats (Zamorano *et al.*, 1995) and in other animals (Skipor *et al.*, 2010). There is a close relationship between the contractility of the arteries and the production of PGs, especially during the estrous cycle (Skipor *et al.*, 2010). There are also some studies on the influence of steroids on the norepinephrine-mediated contractile response (Zamorano *et al.*, 1994; Ok *et al.*, 2014).

Non-steroidal anti-inflammatory drugs (NSAIDs) have a common use in the treatment of fever, pain and inflammation. If we look at the mechanism of action of NSAIDs, they inhibit cyclooxygenase (COX) by pharmacological action which catalyzes to convert the arachidonic acid to PGs (Misko *et al.*, 1995).

In this study, we investigated the effect of indomethacin a potent nonselective inhibitor of PGs synthetase. The enzyme has two isoforms known as COX-1 and COX-2, both located in the endoplasmic reticulum (Mitchell and Warner, 2006). COX-1 plays an important role in routine physiological functions whereas COX-2 shows up in inflammatory reactions and facilitates the cell differentiation (Steinmeyer, 2000). Another isoform of cyclooxygenase called COX-3, has been known to be a variant of COX-1 which is most abundant in the heart and cerebral cortex (Chandrasekharan *et al.*, 2002). Indomethacin inhibits both isoforms as a non-selective COX inhibitor (Centinkaya *et al.*, 2008).

Additionally, indomethacin affects the selective follicle-stimulating hormone peak during the period of ovulation (Shirota *et al.*, 1998). Indomethacin blocks cyclooxygenase, which would affect the contractile response of norepinephrine during the estrous cycle. It is important to note that prostacyclins play an essential role in regulating blood pressure in normotensive and hypertensive animals, among other functions (Mohale *et al.*, 2014). Our results demonstrate that indomethacin produces an inhibition of cyclooxygenase in the concentration of PGE_2 in the aorta in the 4 stages of the estrous cycle (Figure 5).

Vascular complications are a major cause of increased mortality in patients with diabetes mellitus (Farkas *et al.*, 2000). Previous studies have shown alterations in the vascular response of both vasodilators and vasoconstrictors (Meraji *et al.*, 1987). Concerning the cardiovascular system, there has been a strong debate regarding which isoform is involved in the production of vascular prostacyclin. However, it is now widely accepted that only COX-2 is expressed in endothelial cells (Kirkby *et al.*, 2012).

According to our results, although the norepinephrine concentrations versus contraction curves are similar in the absence and presence of indomethacin (Zamorano *et al.*, 1994), it can be observed that at high concentrations of norepinephrine the contraction is of greater magnitude, which confirms that cyclooxygenase produces an increase

in contraction, effect especially evident at the level of proestrus.

It is known that the vascular response to vasoconstrictor hormones can vary considerably depending on the stage of the estrous cycle; some changes in the release of prostanoids by tissues may have some physiological functions including the modulation of vascular sensitivity and regional vascular resistance (McGiff *et al.*, 1976). The effect indomethacin (COX-1/COX-2 inhibitor) demonstrates the sensitivity of the vascular response to PGs, increasing the contraction of the aortic tissue.

Our results are consistent with the literature, where indomethacin has been shown to enhance vasoconstrictor responses to various agents such as norepinephrine and phenylephrine by preventing vasodilatation induced by PGs formation (Kristova *et al.*, 2000).

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