NEONATAL HYPERTHYROIDISM ON RAT HEART: INTERRELATION WITH NITRIC OXIDE AND SEX.

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SUMMARY

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INTRODUCTION

A close relationship between thyroid status and cardiac function has been well established in adult mammals and humans (Purtell et al. 2010; Goumidi et al. 2011; Adamopoulos et al. 2013). Several works have been carried out to pinpoint those important variables whose manipulation in early postnatal life results in long-lasting effects upon cardiovascular function. In this context, the induction of a transient hyperthyroidism in rats at neonatal period lead, at the adult life, to hormonal, neuronal, and metabolic disturbances that may influence the heart function in the adult life (Ratajczak et al. 2005; Rodrigues et al. 2009; Wilcoxon and Redei, 2009; de Picoli Souza et al. 2006). The mechanisms underlying the repercussions at the adult life induced by precocious events at the postnatal period are not fully known. Gerdes et al. have shown that neonatal hyperthyroidism reduced cardiomyocyte proliferation, contractile function and calcium handling albeit by different means (Genders et al 1983; Heron and Rakusan 1996). The neonatal period of mammalian heart development is characterized not only by changes in tissue composition and cardiomyocyte morphology but also by significant growth and maduration of the microvascular network (Heron and Rakusan, 1996). Several studies showed that thyroid hormones and nitric oxide (NO) are involved in many different signaling pathways related with normal postnatal cardiac development and maturation (Pracyk and Slotkin 1992; Ratajczak et al., 2005). Although several authors showed that thyroid axe is regulated by sex steroids; the mechanisms underlying the reported sexual dimorphism are poorly defined (Fichas). Already in 1975 increased circulating estrogen levels were reported in women with thyrotoxicosis, associated with ovulation failure hypothalamic-pituitary level (Akande 1975). We previously reported a functional interaction between thyroid hormones,

endothelial cells and nitric oxide (NO) and we demonstrated that thyroid hormones regulate intrinsic heart rate through a different sympathetic nerve activity involving the nitric oxide pathway (Fellet et al. 2004; 2006). The importance of membrane microdomains such as caveolae as well as caveolins in the control of these signaling pathways has been demonstrated in vessels and myocardium (23, 27). An impaired endothelium-dependent vasodilatation resulting from a reduction in NO availability has been demonstrated in hypothyroidism. We recently showed that thyroid hormones would be one of the factors involved in the modulation of cardiovascular NO production and caveolins tissue-specific abundance regardless of age. Hypothyroidism appears to impact and/or contribute in a differential way to aging-induced changes in the myocardium and aorta tissues in adult life (Sarati et al. 2012). However, little is known about the involvement of this pathway and sex on heart function in neonatal hyperthyroid rats when tested in adulthood.

The aim of the present study was to examine i) if postnatal T_3 treatment affect cardiac function during the second months of life in rats; ii) if postnatal hyperthyroidism alters cardiac NO pathway and iii) if gender influences the neonatal hyperthyroidism-induced cardiac and growth alterations.

METHODS

Animals

All procedures were reviewed and approved by the National Food, Drug and Medical Technology Administration (ANMAT), National Department of Health and Environment, Argentina (No. 6344-96). Pregnant Sprague-Dawley rats were obtained from the breeding

laboratories of the “Facultad de Farmacia y Bioquímica” (Universidad de Buenos Aires, Argentina). They had free access to commercial standard rat chow from Nutrimentos Purina (Buenos Aires, Argentina) and received water ad-libitum. They were housed one animal per cage under controlled humidity and temperature conditions, with an automatic 12-hour light/dark cycle. Male and female Sprague-Dawley rats, weighing approximately 10 g were used in this study. Animals were randomly assigned to one of the two experimental groups: (1) control-or (2) T3- treated group.

Treatment of each group was started on the third day after birth and was conducted as follows:

Control rats (Eut): euthyroid animals who received s.c. injections of 0.9 NaCl (0.1 ml/100 g body weight (BW) every 2nd day during 60 days (Heron and Rakusan, 1996).

T_3-treated rats (Hyper): animals received s.c. injections of triiodotironine (T_3) (Sigma, 20ug/100g BW) every 2nd day during 60 days (Heron and Rakusan, 1996).

Determination of treatment efficacy

Establishment of altered thyroid status was confirmed by measurement of thyroid-stimulating hormone (TSH), triiodotironine (T_3) and total thyroxine (T_4) by radioimmunoassay in serum samples obtained at the experimental end-point (Britton et al. 1975). The TSH kit was provided by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health (Bethesda, USA). Results were expressed in terms of rat TSH standard (rTSH-RP-2). Intra- and inter-assay coefficients of variation for TSH were 8.7% and 13.4%, respectively.

Experimental protocol

Body weights, tail and total lenght of animals were determined at 0 and 60 days post treatment. At the end of experimental period (60 days), animals were anesthetized with urethane (1.0 g/kg, ip), their chests were shaved under aseptic conditions and echocardiographic measurements were performed in the left lateral decubitus position. Two-dimensional directed M-mode images were obtained using a Sonoscape (A6 Vet) system with a 9-4 MHz transducer. Measurements were taken in the right parasternal short axis plane at the level of the mitral valve leaflets. Left ventricle (LV) internal diameter (LVID), LV posterior wall thickness (PWT) and anterior wall thickness (AWT) were measured in both systole (s) and diastole (d). Ejection fraction (EF), fractional shortening (FS) and systolic volume were measured from ventricular internal diameters by the echocardiography system. All determinations were made according to the guidelines of the American Society of Echocardiography. Each rat was then instrumented with catheters. Animals were kept under anesthesia by additional small doses of urethane throughout the experiment. Body temperature was monitored with a rectal probe and maintained at 37.0 ± 0.5 ºC with heating lamps to avoid the influence of temperature on cardiovascular parameters during the experiment. To ensure an open airway, a tracheotomy was performed using polyethylene tubing (3.5 or 4 mm ID, Portex). Mean arterial pressure (MAP), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured through a cannula inserted in the right femoral artery and connected to a pressure transducer (Statham P23 ID, Gould Inst., Cleveland, OH, USA); measurements were recorded with a polygraph (Physiograph E & M Co, Houston, TX, USA) for the duration of the experiment. Heart rate (HR) was derived from the pulsatile pressor signal via tachographic beat-to-beat conversion

with a tachograph preamplifier (Coulbourn Instruments, Inc., tachometer S77-26, PA, USA). The Labtech Notebook program (Laboratory Tech., Wilmington, MD, USA) was used for data acquisition.

Hemodynamic parameters were recorded over a 30 minutes period to allow stabilization of MAP and HR before the start of the experimental protocol. After a 30 minutes stabilization period, basal values were measured over a 5 minutes period (n=15 each group).

At the end of the experimental protocol, rats were sacrificed by overdose of anesthesia and the hearts were removed. Right atria (RA) and LV were isolated. LV weight (LVW) was determined in order to calculate LVW/BW ratio, a parameter of cardiac hypertrophy, which is commonly observed in hyperthyroidism (Kahaly and Dillmann 2005).

Western blot analysis for NO synthase (NOS), caveolin (cav) 1 and 3 proteins were performed in these tissues, and NOS activity was measured according to the method of the conversion of [^{14}C (U)]-L-arginine to [^{14}C (U)]-L-citrulline (Sarati et al. 2012).

NOS activity

Capacity for cardiac NO formation was assessed determining NOS activity in RA and LV from control and T_3-treated rats by measuring the conversion of [^{14}C (U)]-L-arginine to [^{14}C (U)]-L-citrulline. Tissue homogenates (approximately 50 µg protein) were incubated in Tris-HCl buffer (pH 7.4) containing 1 µg/mL L-arginine, [^{14}C (U)]-L-arginine (346 µCi/mL), L-valine (67 mM), NADPH (1 mM), calmodulin (30 nM), tetrahydrobiopterin (5 µM) and $CaCL_2$ (2 mM) for 60 minutes at room temperature. At the end of the incubation

period, the NOS reaction was arrested by addition of a buffer solution containing 20 mM HEPES buffer and 20 mM EDTA, pH 5.5. Reaction mixtures were loaded onto cation exchange columns (Dowex AG 50W-X8, Na+ form; Bio-Rad) and [^{14}C (U)]-L-citrulline was eluted from columns with 0-50 ml ddH_2O. The amount of [^{14}C (U)]-L-citrulline eluted was quantified using a liquid scintillation counter (Wallac 1414 WinSpectral; EG&G Company, Turku, Finland) as described previously (Sarati et al. 2012). All compounds, except [U-^{14}C]-L-arginine monohydrochloride (346mCi/mmol, Amersham Life Science), were purchased from Sigma Chemie. Protein determination was made using the Lowry method, with bovine serum albumin as a standard.

Western blot analysis

The heart samples were homogenized on ice with a Tissue Tearor (Biospec Products Inc.) in homogenization buffer (50 mmol/l Tris, 0.1 mmol/l EDTA, 0.1 mmol/l EGTA, 1% Triton, 1 mmol/l PMSF, 1 µmol/l pepstatin, 2 µmol/l leupeptin, 1x protease inhibitor cocktail from Roche Diagnostics). Protein concentration in the Triton-soluble supernatant was determined using the Lowry assay. Equal amounts of protein (100 µg protein/lane) were separated by electrophoresis in 7.5% SDS-polyacrylamide gels (Bio-Rad, Munich, Germany), transferred to a nitrocellulose membrane (Bio-Rad) and then incubated with rabbit polyclonal anti-NOS and anti-cav antibodies, both diluted at 1:500. The primary antibodies were: polyclonal rabbit anti-inducible NOS (iNOS) (epitope at the carboxy terminus), anti-endothelial NOS (eNOS) (epitope at the amino terminus), anti-neuronal NOS (nNOS) (epitope at the amino terminus), anti-cav 1 (H-97, sc-7875) and anti-cav 3 (H-100, sc-28828). Finally, a secondary immunoreaction with a goat anti-rabbit antibody conjugated with horseradish peroxidase (dilution 1:5000) was performed. Samples were

revealed by chemiluminescence using ECL reagent for 2-4 minutes. Density of the respective bands was quantified by densitometric scanning of Western blots using a Hewlett-Packard scanner and Totallab analyzer software (Biodynamics Corp., Seattle, WA, USA), and protein amounts were calculated by comparison to the densitometric values of the corresponding standard (Towbin et al. 1979). Protein levels were expressed as a ratio of the optical densities of NOS and cav isoforms and β-actin band (using anti-beta actin, clone EP1123Y, rabbit monoclonal antibody) to check for any inaccuracies in protein loading.

Langerdorf Technique

Rats of each groups were anaesthetized with diethylether and heparin (250 IU) was injected into the jugular vein. Hearts were excised quickly and cooled in ice-cold saline until contractions stopped. Hearts were then mounted on a modified Langendorff apparatus (Hugo Sachs Elektronik, March-Hugstetten, Germany) and perfused isovolumically at a constant pressure of 70 mmHg with a non-recirculating Krebs–Ringer bicarbonate solution of the following composition (in mmol/L): NaCl 120; NaHCO3 25; KCl 4.8; MgSO4 1.33; K H2PO4 1.2; CaCl2 1.6; Na2EDTA 0.02; glucose 10. The perfusate was gassed with 95% O2 and 5% CO2 (pH 7.4) and kept at a constant temperature of 37º C. After instrumentation, each heart after a 25-minutes equilibration period was subjected to 25 minutes of global ischemia and 30 minutes of reperfusion (RP) (ischemia was started by shutting off the perfusate flow) (Marina Prendes et al. 2011).

Measurement of heart function

For measurement of LV pressures, the left atrium was removed and a latex balloon connected to a pressure transducer was inserted into the left ventricle through the mitral

valve. The volume of the balloon was adjusted to obtain left ventricular end-diastolic pressure (LVEDP) of 10 mmHg. Values for LV developed pressure (LVDP), peak rate of contraction (+dP/dt) and peak rate of relaxation (-dP/dt) were obtained using a digital data acquisition system (*Unkel Scope Configuration Program for the PCLabCard Data Acquisition Boards from Advantec, USA.* This program was adapted and modified by the technical assistant). HR was measured by means of a counter triggered by the LVDP pulse. Rate-pressure product (RPP) was determined by multiplying HR by LVDP.

Only hearts with LVDP > 60 mmHg and heart rate > 200 bpm at the end of the equilibration period were included in the study (n=8/group) (Marina Prendes et al. 2011).

Materials

The antibodies for the three NOS isoforms (iNOS (610333), eNOS (610298) and nNOS (610311)) were supplied by BD Biosciences and anti β-actin by Millipore (04-1116). Antibodies anti-cav 1 (sc-7875) and 3 (sc-28828) were supplied by Santa Cruz Biotechnology, Inc. and the secondary antibody (170-6515) by Bio-Rad laboratories. The Western Blot Detection System and Hybond-ECL membranes were supplied by Amersham Pharmacia Biotech. Biochemicals were supplied by Sigma Chemical Co (Saint Louis, MO, USA). L-[^{14}C (U)] arginine substrate was supplied by Perkin Elmer Life and Analytical Sciences, Boston, MA, USA. A Wallac 1414 WinSpectral (EG&G Company, Turku, Finland) liquid scintillation counter was used. AG 50W-X8 cation exchange resin was supplied by BIO-RAD Laboratories.

Ethical approval for animal experimentation

Animals were cared for according to regulation 6344/96 of Argentina's National Food, Drug and Medical Technology Administration (ANMAT) and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). Experimental procedures were approved by the ethics committee of the School of Biochemistry and Pharmacy (CEFFB), Universidad de Buenos Aires, Argentina.

Statistical analysis

Data in tables and figures are mean values ± SEM. Data were evaluated with univariate and multivariate approaches for a completely randomized design, with a structure of two factors (sex and thyroid hormones). For each variable, ANOVA or MANOVA analysis was performed when appropriate. The Levene's and Shapiro-Wilk's tests were used to evaluate homogeneity of variances and normality of data, respectively. When normality and homogeneity of variances assumptions were satisfied, the Bonferroni multiple comparison tests were run. In the case of non-homogenous variances, a multiple comparison test, such as Tamhane, was run. To detect association among variables, a correlation analysis was performed and the Pearson coefficient was calculated. All statistical procedures were performed using the SPSS statistical software package version 16.0. Statistical significance was set at $P<0.05$.

RESULTS

Treatment efficacy

Treatment with T_3 OJO PONER TODAS IGUALES was effective in establishing a hyperthyroid state in male as well as female rats. Both gender showed that T_3 treated rats had a lower serum TSH and T_4 levels and higher T_3 levels than age-matched control animals. Female rats had a lower BW and LVW than male animals. Additionally, BW decreased while LVW increased in T_3 treated rats after hormonal treatment in both gender of animals. LVW/BW was similar in both genders of rats. This relationship increased after T_3 treatment (Table 1).

Growth and hemodynamic parameters

We evaluated the relationship between thyroid status and growth in the early stages of the life of the animals. The results show that total and tail length were lower in female rats. T_3 treatment did not modify these parameters neither male nor females rats (Table 2). Table 2 also shows that there were no changes in SBP, DBP, MBP and HR between control male and female rats. Hyperthyroidism increased SBP, MBP and HR in both sex.

Echocardiographic measurements

Table 3 shows no differences in AWT and PWT in systole and diastole, EF and FS between control male and female rats. Female control rats had lower LVID in systole and diastole than male animals. Hyperthyroidism decreased LVID in systole and diastole only in male rats.

Langerdorf Technique

The exposure to 25 minutes no-flow global ischemia led to complete cessation of spontaneous contractions in both control and experimental rat hearts, and HR returned to preischemic values during the 30 minutes RP.

As indicated by RPP, +dP/dt and –dP/dt (Table 4), the left ventricle function reached similar values in female control and T_3 hearts during reperfusion, although the LVEDP was higher at the beginning of reperfusion in the experimental group. On the other hand, male T3 hearts reached lower values in left ventricular function and greater development of LVEDP (Table 4) than male control ones throughout reperfusion.

Nitric oxide synthase in male and female rats.

Figure 1A illustrates total NOS activity from RA in male and female rats. The results show that female euthyroid rats had higher NOS activity than male age-matched animals. Hormonal treatment decreased this enzyme activity in both sex. Western blot analysis revealed that eNOS protein levels were similar in both sex of rats. T_3 treatment did not change these protein levels in male at 60 days, but increased them in female rats (Fig. 1B). Fig. 1C shows that nNOS protein levels were similar in female and male animals. With T_3 treatment, nNOS protein levels did not change. Additionally, iNOS protein levels did not change by gender and thyroid status of the animals (Fig.1D).

Figure 2 shows cav protein levels in RA. At 60 days, there were no differences between male and female animals in cav 1 protein levels. Hyperthyroidism decreased cav 1 protein levels in male and female rats (Fig. 2A). Female rats had higher cav 3 proteins levels than male animals at 60 days. T_3 treatment increased this protein only in male animals (Fig. 2B).

Figure 3A illustrates total NOS activity from LV tissue. The results show that female rats had lower NOS activity than male animals. T_3 treatment increased NOS activity in both sex. Concerning eNOS protein levels, there were no differences between sex at 60 days of life. Hyperthyroidism did not modify this parameter (Fig. 3B). nNOS and iNOS protein levels did not change by gender and thyroid status of the animals (Fig.3C and D). Figure 4 shows cav protein levels in LV. Cav 1 protein levels were lower in female rats than male animals. Hyperthyroidism did not modify protein levels (Fig. 4A). There were no differences between sexes in the cav 3 protein levels. Hyperthyroidism decreased it in both sex (Fig. 4B).

DISCUSSION

Hyperthyroidism affects women 5–10 times more frequently than men (Jansson et al. 1993). Simple clinical features, such as gender and age may affect both disease presentation and predict response to treatment of this hormonal disorder. There is increasing evidence suggesting that adaptations to the fetal environment "program" physiological changes in the adult. In this study, T_3 treatment was effective to establish hyperthyroid state as shown increased serum T3 and reduced TSH and T4 levels in male and female rats. This hyper functional state was also evidenced by decreased BW as well as increased LVW and LVW/BW observed in T_3-treated animals. In this study, the changes in the thyroid axe were similar in both genders. Different neurohormonal factors (nombrar algunos???) would be involved in normal growth during the neonatal period of development relacionados con la function tiroidea en diferentes sexos. (fichas) Los resultados son controversiales vinculados

con la function cardiaca. Several authors showed that some of alterations of thyroid axe seem to be gender related in humans (Samuels 1998) and in rats (Greeley et al. 1982). In this context, Correa da Costa and Rosenthal suggested that aging may affect thyroid function and regulation differently depending on gender, and thus be modulated by gonadal hormones (Correa da Costa and Rosenthal 1996). The increased T4 levels in female rats could result, in large part, from increased estrogen levels. However, our findings no concuerdan con lo demostrado por estas investigaciones. Already in 1975 increased circulating estrogen levels were reported in women with thyrotoxicosis, associated with ovulation failure hypothalamic-pituitary level (Akande 1975). Moreover, it is interesting to note that hormonal treatment induced a higher increase in LV mass in female animals than male. This finding could indicate that the growth mechanism governing the elicited cardiac growth response to T_3 treatment differs between female and male rats. Several studies showed that treatment with thyroid hormone during neonatal period of development not only leads to accelerated cardiac growth but also influences cardiac muscle cell proliferation and consequently cardiac muscle cell numbers (Dubeck et al., 1989; Gerdes et al., 1983). Abnormal growth of the heart may have a long-term positive or negative impact on cardiac function in the adulthood. By contrast, the findings of our study agreed with careful analysis of the Framingham Heart Study data that has shown that LV mass is significantly greater in men than in women (Pearce et al. 2010). It is well known that hyperthyroidism induced experimentally by administration of T_3 and T_4 has profound effects on cardiovascular function (Fazio et al. 2004; Vargas et al. 2006; Brandt et al. 2011). Gay and coworkers showed increases in HR, RVP, LVP, ventricular contractility, and mean circulatory filling pressure in male Sprague-Dawley rats treated for 10–12 days with T_4 (Gay et al. 1988). On the other hand, no differences in body growth parameters between

sexes neither nor hormonal treatments were observed in this study. Si bien nuestros resultados no estarían de acuerdo con los diferentes hallazgos, y pensando un factor como el NO podría estar involucrado, estudiamos el sistema de NO. This would suggest that other neurohormonal factors would be involved in normal growth during the neonatal period of development (Hasegawa et al. 2001; Haddad et al. 2008; Mizuno et al. 2008). IN first step, Results from the present study indicate that as adult, hyperthyroidism during the neonatal period induces positive chronotropic and inotropic effects on the heart that would be associated with NO system as was previously reported by us in a hypothyroidism model (Sarati et al. 2012)..

In the atria, female animals had higher NOS activity than male rats. However, these animals had similar HR values. Female animals would have another bradycardic factor involved in this effect. The increased NOS activity of female rats was associated with increased cav3 protein levels. Thus, the rise of enzyme activity would be due to another regulator of the enzyme. Hyperthyroidism decreased this activity in both sexes meanwhile HR increased. This changed was more pronounced in female than male animals. Thus, hyperthyroid-induced tachycardia may be due to low NO production. However, we cannot throughout the involvement of sympathetic nervous system. Hyperthyroid state also induced increased cav 3 and decreased cav1 protein levels in male animals. In female rats, the decreased NOS activity induced by hyperthyroidism was accompanied by a rise in eNOS protein levels and decreased in cav 1. These findings suggest that eNOS protein would be not a functional enzyme and other modulators of the enzyme would be relevant in this model of hyperthyroidism. The electrocardiographic studies confirmed the

chronotropic effect but no abnormalities were observed in the electrical conduction, nor any sign of arrhythmia or atrial fibrillation in hyperthyroid rats.

In the LV, female animals had a lower NOS activity than male rats (ARREGLAR EL GRAFICO DE BARRAS PORQUE ESTA EQUIVOCADO EN LA FIGURA). This lower NO production of female animals was associated with a lower internal ventricle diameter in systole and diastole and similarities of cardiac function. Additionally, hyperthyroidism increased this activity in both sex at 60 days. The changes in NOS activity induced by hyperthyroid state would be due to a lower cav 3 protein levels in both gender. Furthermore, the echocardiographic measurements showed a significant increase in diastolic and systolic diameter in hyperthyroid rats compared to the control group. No change was observed in the thickness of the anterior and posterior wall. Hormone treatment did not cause changes in any of the parameters evaluated by echocardiography in female animals. Langerdorf technique revealed that hearts from female rats treated either controls recover to the ischemia better than male animals. ESTO SUGIERE ……..

Our data indicate that the myocardium of male and female rats undergoes distinct post-pubertal changes that confer to the latter a relatively stronger adaptation profile that appears to be related to ability of the myocytes to generate NO. Others studies confirmed these hypotheses. Previous experimental studies reported that the heart of sedentary male rats displayed greater stroke work and volume than the heart of female rats (Schaible et al. 1981) and further advance our knowledge related to genderspecific adaptations of the heart muscle to physiological stimuli such as exercise and to pathological stimuli such as myocardium loss due to myocardial infarction, a problem that results in morbidity and

mortality in a large percentage of the human population (Schaible et al. 1984; Scheuer et al. 1987).

Results of this study demonstrating clinically relevant gender-based differences in the pathophysiology of hyperthyroid heart have raised new questions regarding the mechanisms responsible for the observed differences. These studies suggest that gender-related intrinsic factors may modulate the response to pathophysiological factors that lead to cardiovascular dysfunction.

We hypothesized that the combined effects of hyperthyroidism and perinatal age would have a more deleterious effect on the cardiovascular system of male than female rats, because development associated is not tolerated as well in male as in female rats, and this should be further exacerbated by the superimposition of hyperthyroidism, leading to heart failure.

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Table 1. Biological variables in male and female rats.

Animal´s group		TSH (ng/ml)	T_3 (ng/ml)	T4 (µg/ml)	BW (g)	LVW (mg)	LVW/BW (mg/g)
Male	Con	1.40±0.01	1.20±0.01	2.50±0.01	323±7	747±3	2.30±0.03
	Hyper	0.40±0.01*	2.40±0.02*	0.80±0.04*	287±7*	773±2*	2.70±0.05*
Female	Con	1.42±0.01	1.20±0.01	3.40±0.01**	217±5**	484±3**	2.20±0.04
	Hyper	0.50±0.02*	2.60±0.02*	1.10±0.03*	201±3*	615±4*	3.01±0.02*

Data are mean±SEM; n=10; *p<0.05 versus Con; **p<0.05 versus male rats. Con (control rats); Hyper (hyperthyroid rats), TSH (thyroid stimulating hormone) level; T_3 (total triiodothyronine) level; T_4 (total thyroxine) level; BW (body weight); LVW (left ventricular weight); LV/BW (rate left ventricular weight/ body weight).

Table 2. Growth and hemodynamics parameters in male and females rats.

Animal´s group		Tail (cm)	Total (cm)	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)	HR (bpm)
Male	Con	18.0±0.1	41.0±0.2	97±3	63±2	70±2	322±3
	Hyper	17.0±0.2	40.0±0.2	115±1*	65±2	81±1*	408±2*
Female	Con	16.0±0.2**	37.0±0.4**	101±2	61±3	74±2	311±9
	Hyper	16.0±0.2**	36.0±0.2†	121±3* **	67±3	84±4*	429±5* **

Data are mean±SEM; n=10; *p<0.05 versus Con; **p<0.05 versus male rats. Con (control rats); Hyper (hyperthyroid rats), SBP (systolic blood pressure); DBP (diastolic blood pressure); MBP (mean blood pressure); HR (heart rate).

Table 3. Echocardiographic data.

Animal´s group		AWTd (mm)	AWTs (mm)	LVIDd (mm)	LVIDs (mm)	PWTd (mm)	PWTs (mm)	EF (%)	FS (%)
Male	Con	1.8±0.1	2.9±0.1	5.8±0.2	2.8±0.1	2.1±0.2	2.9±0.1	86±3	52±3
	Hyper	2.0±0.1	2.8±0.1	4.6±0.3*	2.2±0.2*	2.2±0.2	3.1±0.1	87±2	46±3
Female	Con	2.0±0.1	2.8±0.1	4.2±0.1**	2.4±0.2**	2.0±0.1	2.6±0.1	80±4	45±4
	Hyper	1.60±0.03* **	2.7±0.1	4.3±0.3**	2.6±0.3**	1.9±0.1	2.8±0.1	86±4	51±4

Data are mean±SEM; n=10; *p<0.05 versus Con; **p<0.05 versus male rats. Con (control rats); Hyper (hyperthyroid rats), LV (left ventricle); LVIDs (LVinternal diameter in systole); LVIDd (LV internal diameter in diastole); AWTs (anterior wall thickness in systole); AWTd (anterior wall thickness in diastole); PWWs (posterior wall thickiness in systole); PWTd (posterior wall thickness in diastole); EF (ejection fraction); and FS (fractional shortening).

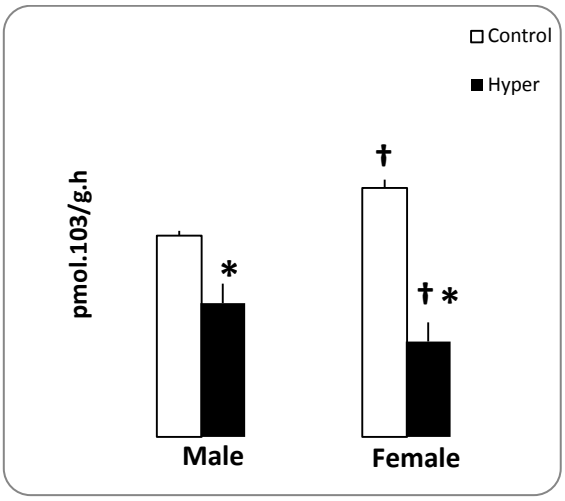
Table 4. Langerdorf measurement. (falta rango)

		Reperfusión		
		5 (min)	15 (min)	30 (min)
P x F (%)	M C	14.14±	72.27±	78.14±
	M T_3	7±	26.75±	50±
	F C	16.86±	54.57±	66.86±
	F T_3	23.03±	58.3±	68.2±
+dP/dT (%)	M C	67.00±	77.29±	79.71±
	M T_3	17.00±	53.75±	69±
	F C	30±	77.67±	77.11±
	F T_3	55.50±	60.50±	67.50±
-dP/dT (%)	M C	61.83±	83.17±	81.00±
	M T_3	21.50±	70.75±	43.25±
	F C	45.25±	80.25±	66.25±
	F T_3	47.00±	50.33±	51.83±
LVEDP (%)	M C	13.75±	4±	0
	M T_3	29.75±	18±	0
	F C	27±	1±	0
	F T_3	40±	13.5±	0

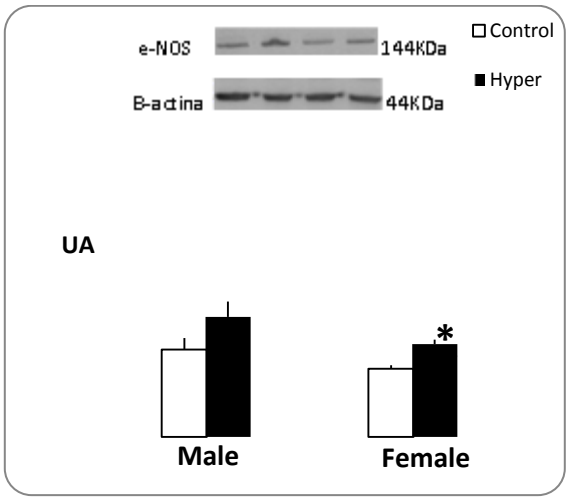
Data are mean±SEM; n=8; *p<0.05 versus Con; **p<0.05 versus male rats. Abreviaturas

Figure1. Atrial tissue.

A



B



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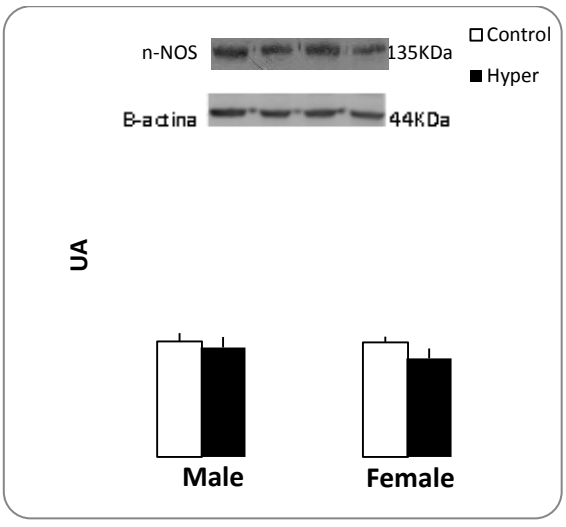

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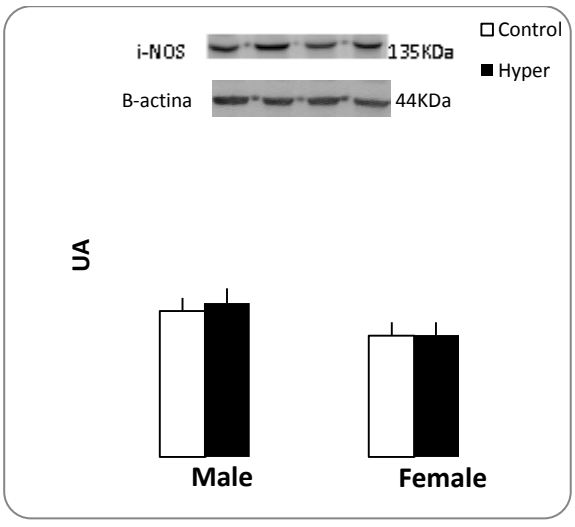
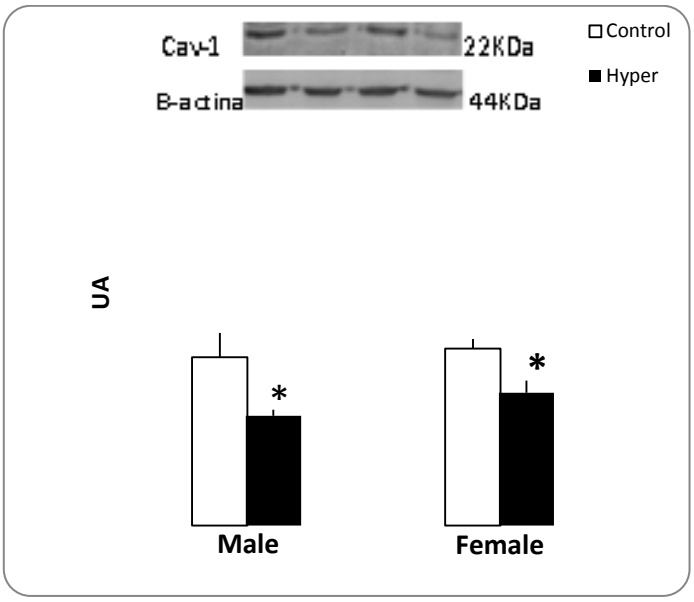


Figure 2 . Caveolins and atrial tissue.

A



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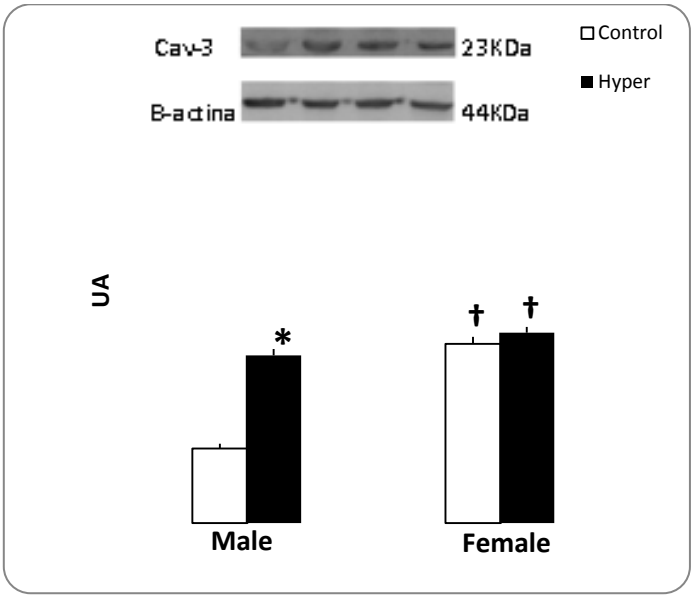
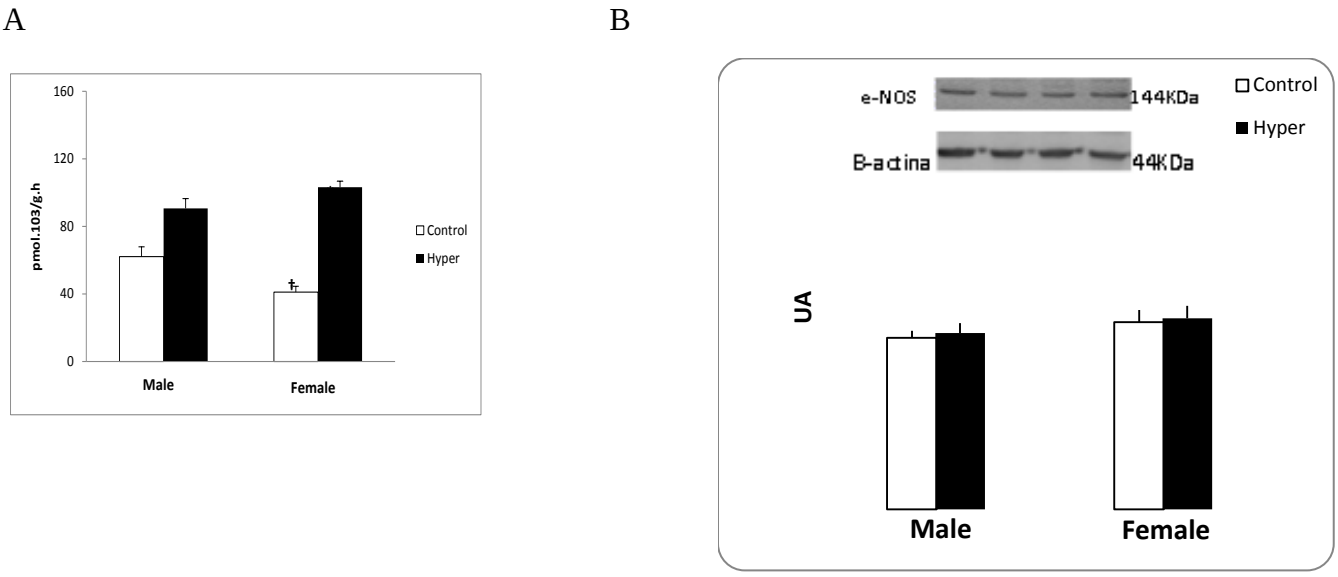


Figure 3. NOS and ventricle tissue.



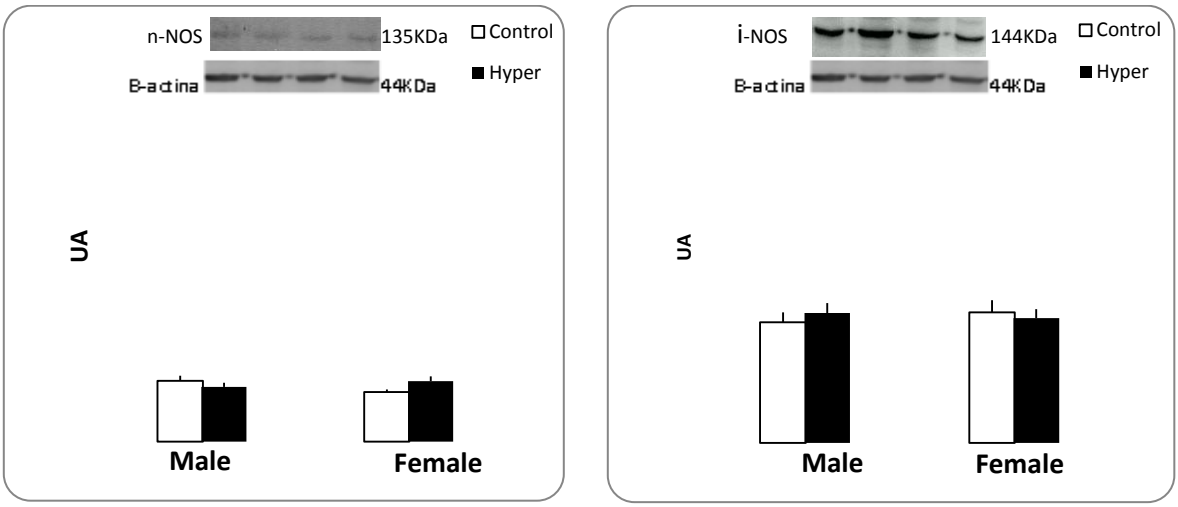
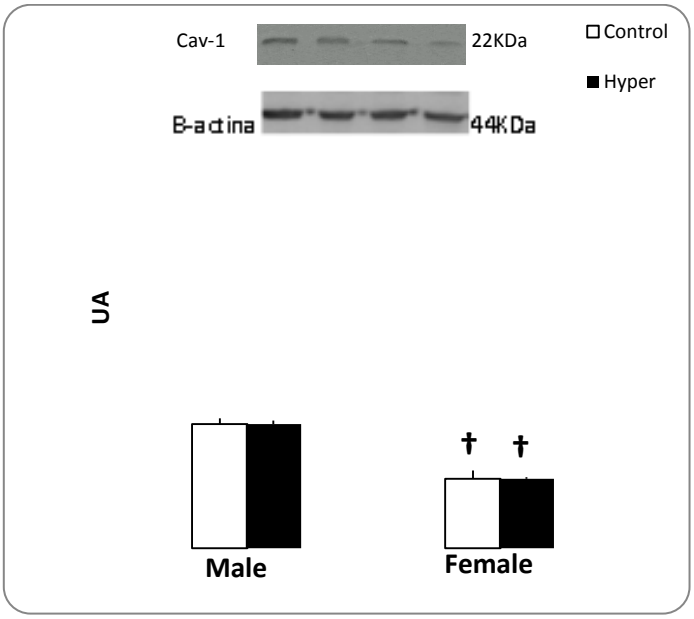
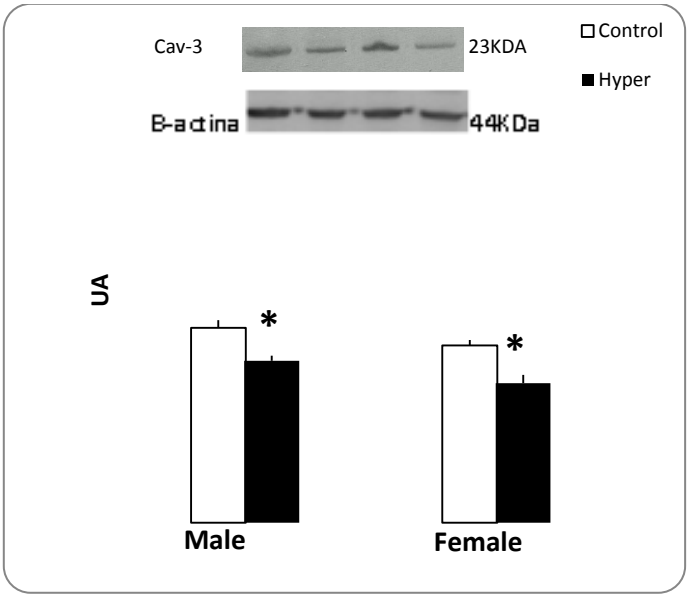


Figure 4. Caveolins and ventricle tissue

A


Cav-1
22KDa
Control
Hyper
B-actina
44KDa
UA
†
†
Male
Female

B


Cav-3
23KDA
Control
Hyper
B-actina
44KDa
UA
*
*
Male
Female