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## PULMONARY HYPERTENSION MODIFIES RESPONSIVENESS OF SINOATRIAL CELLS OF RAT HEARTS TO ADRENOMIMETICS AND ACTIVATORS OF ATP-SENSITIVE K<sup>+</sup> CHANNELS IN A GENDER-DEPENDENT WAY

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The aim of this paper was to find out how pulmonary arterial hypertension (PAH) affects heart rate in males and females rats. Additionally, the concentration of tumor necrosis factor alpha (TNF-alpha) was monitored. Male and female rats were treated by monocrotaline (*i.p.*, 60mg/kg B.W.) or by equivalent volume of normal saline solution (n=40) and after 4 weeks right auriculae containing sinoatrial cells have been isolated and examined. We have measured heart rate of spontaneously beating right auriculae and serum level of TNF-alpha. In females with PAH, isoprenaline curve was shifted to the left (pD<sub>2</sub> increased from 10,9±3 to 15±3, n=10, P<0,05), but in males with PAH maximal acceleration of beating rate induced by isoprenaline decreased from 175±10 to 146±8 beats/min, P<0.05). Additionally, reduction in heart rate induced by rilmakalim was more significantly decreased in females (from -89±7 to -49±3 beats/min, n=10, p<0.01) than in males (from -68±6 to -46±4 beats/min, P<0.05) with PAH. We did not detect any changes in TNF-alpha in any experimental group. Our results imply that pulmonary hypertension increased sensitivity of sinoatrial cells to isoprenaline and decreased efficacy of rilmakalim to reduce heart rate more significantly in females than in males.

**Key words:** *pulmonary arterial hypertension, rats, monocrotaline, gender, beta adrenergic receptors, ATP-sensitive potassium channels*

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### INTRODUCTION

Pulmonary arterial hypertension (PAH) is a pathological state associated with several cardiovascular diseases. Usually, it is a direct consequence of congenital heart failure and leads to progressive right ventricular failure and death. There are some collagenosis (*e.g.* sarcoidosis), vascular diseases, thrombosis and embolism and some other pathological situations resulting in significant increase of pressure in the pulmonary circulation (1, 2). Moreover, the effects of reversible hypertension appearing during cardiosurgical treatment on post-treatment outcomes have been analyzed (3). Although,

therapeutic strategy in case of definitely recognized PAH is already well established (sildenafil, iloprost, bosentan), it is not yet satisfactory in all patients and prognosis is bad in majority of patients with irreversible, congenital heart abnormality-related PAH (4). In this study we used classical monocrotalin model of PAH in rats and looked for sinoatrial cells function in males and females animals in order to find out possible changes in basal heart rate and role of adrenergic receptors and ATP-sensitive K<sup>+</sup> channels under such a condition. Moreover, previous research has shown higher expression of some inflammatory cytokines by PAH (especially TNF-alpha) in patients after cardiopulmonary bypass (5, 6). Therefore, our

intention was to investigate heart rate changes of isolated, spontaneously beating sinoatrial cells, obtained from males and females rats, belonging to the control or PAH groups. Moreover, we used the pharmacological tools able to stimulate (isoprenaline,  $\beta$ -adrenoceptor agonist) or inhibit (rilmakalim, activator of ATP-sensitive  $K^+$  channels) heart rate. Likewise, we have measured TNF-alpha concentration in serum of all examined groups of animals.

## MATERIAL AND METHODS

All experiments were performed on males and females guinea pigs kept under standard laboratory conditions, at 22-24°C temperature, humidity 50-55%, 12h/12 h light-dark periods and with food and tap water *ad libitum*. Experimental procedures were in accordance with "European convention for the protection of vertebrate animals used for experimental and other scientific purpose" (Council of Europe No. 123, Strasbourg, 1985) and approved by local Ethics Committee.

### Experimental procedures

All animals were divided in four groups: control males (CM), males treated by monocrotalin (MM), control females (CF) and females treated by monocrotaline (MF). The model of pulmonary hypertension (PAH) used in this study is very well known as monocrotalin-induced PAH. After 4 weeks of pretreatment with monocrotaline (one application, *i.p.*, 60mg/kg B.W.) animals were sacrificed, spontaneously beating right auricula were carefully dissected from thorax and attached to isometrical

transducer (K-30, Hugo Sachs Elektronik, Germany) for further examination. In the groups treated by monocrotaline we observed enlarged heart, with the symptoms of congestion in lung-what served as a confirmation of developed PAH. All signals were recorded for further evaluations. Basal heart rate, effects of isoprenaline and effects of rilmakalim were measured. After 40 to 60 min of incubation and stabilization of heart rate ( $300 \pm 30$  beats/min) in Krebs-Henseleit solution aerated by 95% $O_2$ /5% $CO_2$ , kept at 37°C, isoprenaline was added in increasing concentrations, from  $10^{-9}$  M to  $3 \times 10^{-6}$  M, every 2 min, associated with measurement of heart rate, up to the obtainment of the maximal effect ( $E_{max}$ ). Then,  $3 \times 10^{-6}$  M (3  $\mu$ M) of rilmakalim was added and after 5 min heart rate was measured again.

After dissection of right auricula, a sample of blood was taken for later determination of TNF-alpha. After centrifugation (3000/min), serum was separated and kept under -70°C up to the moment of measurement. We used commercial rat TNF alpha ELISA kit.

### Drugs

Isoprenaline was purchased from Sigma, St. Louis, MO, USA, Rilmakalim was a gift from dr. Heinrich C. Englert, Aventis, Germany. The drugs were dissolved in distilled water.

### Statistical analysis

All data are expressed as mean  $\pm$  standard error (S. E.). Comparison and evaluation of data from more than two independent groups have been done by two-way ANOVA with multiple comparison Neuman-Keuls test. A paired Student's *t*-test was

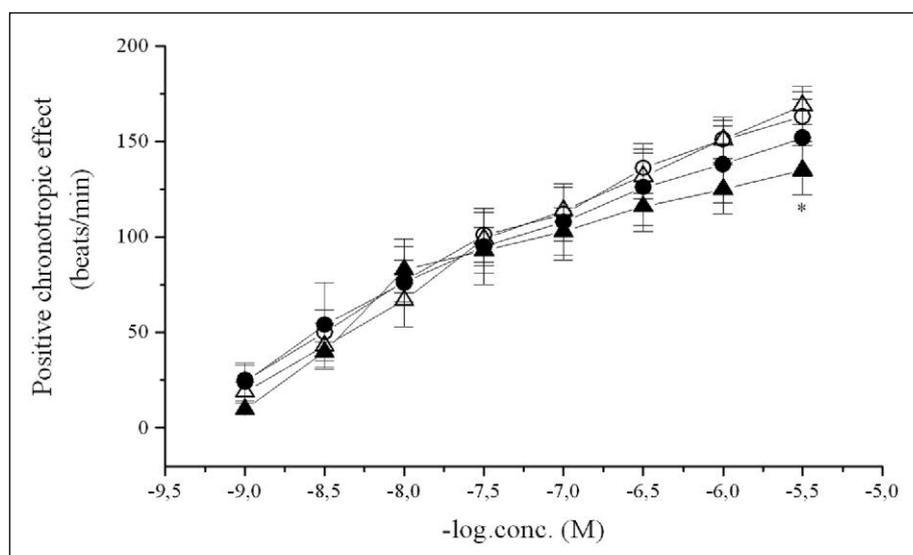


Fig. 1. Concentration-response curves for isoprenaline positive chronotropic effects in control and monocrotaline-treated groups of males and females rats. \* $P < 0,05$ ; significant difference regarding corresponding values in control group. Student *t*-test paired data,  $n = 9-12$ .

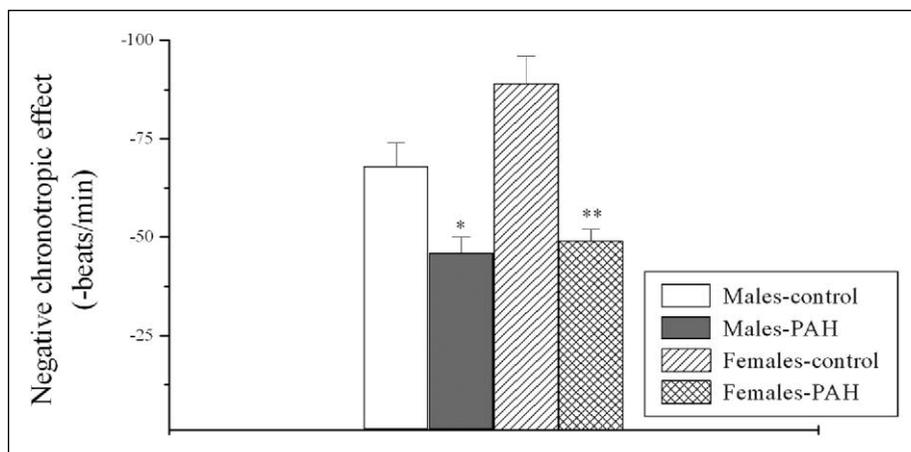


Fig. 2. Negative chronotropic action of rilmakalim at concentration of 3  $\mu$ M produced in preparations of right atria previously stimulated by isoprenaline. The results are expressed as the number of beats by which rilmakalim reduced heart rate. \* $P < 0,05$ ; \*\* $P < 0,01$ ; significant differences in comparison with control groups. Two-tail ANOVA with Neuman-Keuls test,  $F = 14,43$ ;  $n = 10-12$ .

used for comparison of the responses before and after drug administration. A difference of  $p < 0.05$  was considered statistically significant.

## RESULTS

### *The effects of monocrotaline*

Animals treated by monocrotaline (60 mg/kg B.W. *i.p.*) started to change its behavior after about 2 weeks since drug application. They moved slowly, have some skin changes. After 4 weeks, all animals were sacrificed and hearts were removed and carefully examined to confirm signs of pulmonary hypertension. Typical changes, as enlarged right ventricles, and edema signs in lungs confirmed pathological changes related to PAH in monocrotaline groups.

### *Positive chronotropic action of isoprenaline on isolated right auricula in control and PAH groups of females and males rats*

Resting heart rates were similar in all experimental and control groups within range of  $300 \pm 30$  beats/min (data not shown). However, concentration-response curve to isoprenaline (concentration range from  $10^{-9}$  M to  $3 \times 10^{-6}$  M) regarding its chronotropic action was significantly shifted to the left in monocrotaline-treated female group (MF), with the values of  $pD_2$  ( $-\log EC_{50}$ )  $10,9 \pm 2,6$  in CF and  $15 \pm 3$  in MF ( $P < 0.05$ ,  $N = 9$ ). Additionally, maximal efficacy of isoprenaline significantly decreased in MM group (Fig. 1).

### *Negative chronotropic action of an activator of ATP-sensitive K channels, rilmakalim*

Rilmakalim strongly decreased heart rate previously maximally accelerated by isoprenaline in both control groups, males and females, without any

statistically significant difference between them. However, pulmonary hypertension decreased ability of rilmakalim to reverse isoprenaline positive chronotropic action, especially in females (by 56%,  $n = 10$ ,  $p < 0.01$  vs females control, in comparison with males 32%,  $n = 12$ ,  $P < 0.05$  vs. males control) with PAH (Fig. 2).

### *Serum TNF-alpha concentration*

We did not detect any measurable level of TNF-alpha in all examined animals included control males, monocrotaline-treated males, control females and monocrotaline-treated females groups (data not shown).

## DISCUSSION

The main results of this study are hypersensitivity of  $\beta$ -adrenergic receptors to isoprenaline and diminished responsiveness to the activation of  $K_{ATP}$  channels by rilmakalim of the sinoatrial (pacemaker) cells obtained from the hearts of female rats with PAH. The model of pulmonary arterial hypertension (PAH) adopted here is well established and widely used (8). In order to confirm pathological changes, we carefully examined every animal treated by monocrotaline before inclusion in experiments. Heart rate variability under such a condition was already reported, however, the mechanism underlying that arrhythmias was not clarified (9-11). Adrenergic system and  $K_{ATP}$  channels are of great importance in regulation of heart rate and could be modified by hemodynamic changes during development of PAH. We have recently shown that estrogen increased sensitivity of  $\beta_1$  receptors to catechol amines in guinea pigs (12). Sex-related differences in heart function, especially under pathological condition, are well known, and

generally indicate better heart performance in females than in males with heart failure (13). Similar as with humans, female sex seems to exert cardioprotective effects also in animals (14). Detailed analysis of possible sex-differences in rats revealed that female hearts expressed more Cav1.2, RyR and NCX proteins than in males, however no differences were detected in expression of  $\beta$ -adrenoceptors and response to isoprenaline (15). It is in accordance with our results, as we also have not detected any difference in sensitivity to isoprenaline in control groups. However, the differences appeared in females with pulmonary hypertension. It seems that PAH induced redistribution and changes in signaling pathways coupled to  $\beta$ -adrenoceptors, probably due to sympathetic overactivity (16). Thus, leftward shift of the isoprenaline concentration-response curve in female group with developed PAH suggests up-regulation of the population of  $\beta$ -adrenoceptors. On the other hand, significant attenuation of rilmakalim ability to activate ATP-sensitive  $K^+$  channels in females with PAH could be explained by recently published data. Namely, estrogen increases expression of SUR2B subunits of ATP-sensitive  $K^+$  channels, which can bind a ligand (17). This process seems to be accentuated under circumstances of pulmonary hypertension as an adaptive reaction to the gradually increasing pressure and could be one of the key phenomenon for explanation of arrhythmogenesis in females with PAH. Additionally, the role of TNF-alpha in observed pathological changes during development of PAH deserves to be discussed. Previously published data are controversial. Elevated level of this cytokine was detected in myocardium and serum in humans with heart failure and proved to be necessary for hypoxia-related right ventricle hypertrophy (18-20). However, in monocrotaline-model of PAH in rats blockade of TNF-alpha receptors did not affect pulmonary hypertension, what is in accordance with our results (lack of any detectable level of this cytokine in serum) (21). It is possible that earlier reported increase of TNF-alpha in patients with PAH after cardiopulmonary bypass procedures (5, 6) was rather due to surgical manipulation than development of PAH. However, we did not measure TNF-alpha concentration in myocardium and can not exclude some changes of it in the heart muscle.

Taking all together, our data imply that PAH leads to substantial changes in function of females sinoatrial cells, inducing hyper-sensitivity of  $\beta$ -adrenoceptors (females) and blunted responsiveness of ATP-sensitive  $K^+$  channels to the openers in this area of heart, which could be important for explanation of

some proarrhythmic mechanisms under such a condition.

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