ELSEVIER

International Journal of Cardiology 110 (2006) 22 – 26

International Journal of Cardiology

www.elsevier.com/locate/ijcard

Selective inhibition of pinacidil effects by estrogen in guinea pig heart

Ivan Kocić *, Marta Gruchała, Jacek Petrusewicz

Department of Pharmacology, Medical University of Gdańsk, Dębowa 23, 80-204 Gdańsk, Poland

Received 25 March 2005; received in revised form 13 June 2005; accepted 18 June 2005
Available online 10 August 2005

Abstract

Background: Recently, gender related differences in heart function have been extensively studied. Some of them, as differences in repolarization between males and females have been explained by direct effect of estrogen on delayed rectifier K^+ channels and Ca^{2+} channels. It seems that estrogen induces overexpression of SUR2A subunits of ATP-sensitive K^+ channels. The aim of this paper was to compare heart rate changes in male and female guinea pigs in the presence of different potassium channel openers (PCOs).
Methods: We used spontaneously beating right atria from control and estrogen receptor modulator-treated male and female guinea pigs (17-β-estradiol as a stimulator and tamoxifen as a blocker of estrogen receptor located in heart muscle).
Results: In control females, rilmakalim and diazoxide, but not pinacidil elicited concentration-dependent decrease of heart rate. On the other hand, all three PCOs induced similar negative chronotropic action in hearts obtained from male control group (E_{max} was between -40 and -70 bpm, respectively). After two weeks of treatment with 17-β-estradiol, pinacidil failed to significantly decrease heart rate in males however, tamoxifen-pretreated female group responded by decrease in automatism in the presence of rising concentration of pinacidil ($E_{max}=-45\pm6$ bpm, not significantly different from E_{max} in male control $=-40\pm5$ bpm, $n=7$). Interestingly, we observed lower blood concentration of the heart form of lactate dehydrogenase (H-LDH) in female than in male control group. Moreover, H-LDH concentration increased in tamoxifen-pretreated female group and decreased in 17-β-estradiol-treated male group.
Conclusion: Our results indicate that estrogen downregulates H-LDH production and specifically modulate pinacidil action in guinea pig right atria, probably by changes of binding site for this drug in SUR2A receptor, but not for rilmakalim and diazoxide.

Keywords: Estrogen; Heart; ATP-sensitive K^+ channels; Pinacidil; Lactate dehydrogenase

1. Introduction

There are several differences between female and male heart physiology. For instance, females have higher basal rate of heart beating, longer $Q-T$ interval and lower current density of rapid and slow components of delayed K^+ rectifier currents [1–3]. However, there is no sufficient information regarding possible gender differences in function of ATP-sensitive K^+ channels (K_{ATP}), which are unique ion channels that detect metabolic changes in the heart myocytes during ischemia [4–6]. It has been shown recently that pinacidil, a cyanoguanidine potassium channel opener (PCO), produced a negative chronotropic action in male guinea pigs. However, the action in female guinea pigs was only produced during the fall and winter periods, when no regular estrus cycles appeared (less then 1 cycle per month) [7]. To elucidate the mechanism of these findings we designed this study to investigate and compare the functions of K_{ATP} channels in male and female guinea pigs under different experimental conditions using 17-β-estradiol as a stimulator and tamoxifen as a blocker of estrogen receptors. Additionally, we measured concentration of total lactate dehydrogenase (T-LDH) and heart form of LDH (H-LDH) in the blood of the animals used in the experiments.

* Corresponding author. Tel.: +48 58 3491823; fax: +48 58 3491811.
E-mail address: ikocic@amg.gda.pl (I. Kocić).

0167-5273/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ijcard.2005.06.033

2. Material and methods

2.1. Animals

We used female and male guinea pigs weighing between 300 and 500 g, that were kept under standard experimental conditions (humidity 50–55%, 12 h dark and 12 h light periods, temperature 22–24 °C) in accordance with international ethical standards accepted by Ethics Committee of Medical University of Gdańsk, Poland.

2.2. Experimental procedure

Experiments were performed during spring and summer periods. Female guinea pigs were divided into a control group and an experimental group pretreated by tamoxifen. Tamoxifen, an estrogen receptor antagonist, was given i.p., at a dose 0.133 mg/100 g of body weight. Male guinea pigs were divided into a control and an experimental group, treated by 17-β-estradiol at a dose 0.132 mg/100 g of body weight (we also used dose 0.066 mg/100 g B.W. in our pilot study, but this dose was without significant effects on pinacidil action—see results for details). Both drugs have been applied everyday at 8 a.m. for a period of 2 weeks. Applied doses are chosen in accordance with the literature data [8–10]. At the same time the animals from the control group have obtained a same volume of saline and solvent used with the drugs, i.p. The estrus cycle of the females was determined by observation of their behaviors and vulva and vaginal membranes. Opening of the vaginal membrane, reddening of the vulva and nervousness are well known symptoms of the estrus [11,12]. Chronotropic effects have been studied on isolated, spontaneously beating right atria. Detailed procedure of preparation and solution was previously described [13]. If the rate of the beating was regular and stable (usually about 180±20), experiments were continued. After about 45 min of incubation, pinacidil was added in increasing concentrations and the heart rate was measured by isometric transducer F-30 (HSE, Germany) and recorded by a pen-recorder (Cole-Parmer International, USA). If pinacidil did not evoke any significant effect, rilmakalim, another PCO with different structure than pinacidil, was added. Separate series of experiments were performed with diazoxide to confirm previously obtained data in our laboratory [7].

2.3. Lactate dehydrogenase (LDH) assay

Total and heart form of LDH activity were measured in serum obtained from the blood of all experimental groups, by automatic spectrophotometer analyzer (Hitachi 911, Boehringer Mannheim, Germany) at 340 nm wavelength using a phosphate buffer at pH 7.5.

2.4. Drugs

Tamoxifen, estrogen, pinacidil and diazoxide were purchased from Sigma, St. Luis, USA. Rilmakalim was a gift from Dr. Englert, from Aventis Pharma, Frankfurt, Germany. Tamoxifen and estrogen were dissolved into ethanol, while pinacidil, diazoxide and rilmakalim were dissolved into dimethylsulfoxide (DMSO). Ethanol alone was given to the control animals at the same volume (0.05 ml/100 g) as given to the animals treated by tamoxifen or estrogen. The concentration of DMSO in the bath solution was less than 0.1% and had no influence on the heart rate.

2.5. Statistical analysis

Two-way ANOVA with Newman–Keuls multiple comparison test was used to determine statistical significance between means±standard errors (SEM). $P<0.05$ was considered as statistical significant difference. Concentration–response curves and their parameters (slope and pD_2 are calculated by a computer program according to Tallarida and Murray [14].

3. Results

3.1. Effects of potassium channel opener pinacidil on heart automatism in male guinea pigs

Pinacidil evoked concentration-dependent slowing of heart rate (HR). The maximal effect (−40±5 bpm, $n=7$) was observed at 300 μM (Fig. 1). Control rate of beating in the absence of pinacidil (basal rate) was 186±3, $n=7$ and

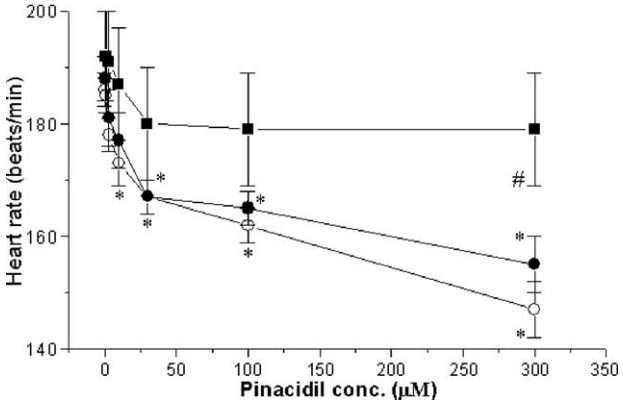

Fig. 1. Influence of 17-β-estradiol on negative chronotropic action of pinacidil in male guinea pigs' right atria. Open circles—control; closed circles—after treatment with 0.066 mg/100 g B.W. of 17-β-estradiol; closed squares—after treatment with 0.132 mg/100 g B.W. of 17-β-estradiol; *P, 0.05, differences related to corresponding control values; #$P<0.05$, difference between maximal negative chronotropic action of pinacidil in control and 17-β-estradiol-treated male guinea pigs. Means± SEM, $n=7$.