Toxic Effects of Palladium Compounds on the Isolated Rat Heart

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Abstract: Taken into consideration limited data about effects of palladium on cardiovascular system, the aim of our study was to compare toxicity of inorganic and organic palladium compounds on the isolated rat heart. The hearts (total number *n*=30, 6 for each experimental group) excised from Wistar albino rats, male sex, age 8 weeks, and body mass 180-200 g, were retrogradely perfused according to the Langendorff technique at constant perfusion pressure (70 cm H_2O). After the insertion of sensor in the left ventricle, the parameters of heart function: maximum rate of left ventricular pressure development (dP/dt max), systolic left ventricular pressure (SLVP), diastolic left ventricular pressure (DLVP), mean blood pressure (MBP) and heart rate (HR)), were continuously registered. The experiments were performed during control conditions, and in the presence of perfusion with incresing concentration of the following: (triethanolamine (TEA), triethanolamine acetate (TEAA), palladium(II)chloride ($PdCl_2$), and *trans*-dichlorobis(triethanolamine-*N*)palladium(II) complex (*trans*-[$PdCl_2$(TEA)2])) started every 30 minutes (30, 60, 90, 120 minute). dP/dt max was not affected significantly by either TEAA, TEA, $PdCl_2$ or Pd complex. SLVP was, also, not affected significantly by either TEAA, TEA, $PdCl_2$, or Pd complex. DLVP was significantly decreased by both TEAA and $PdCl_2$, while TEA and Pd complex did not show significant effect. MBP was significantly decreased only by $PdCl_2$, while TEAA, TEA and Pd complex did not show significant effect. HR was significantly decreased by all compounds- $PdCl_2$, TEAA, TEA and Pd complex. In our study, inorganic palladium compound ($PdCl_2$) induced clear depression of the isolated rat heart contractility, manifested as drop in diastolic and mean blood pressure , and as decrease of the heart rate. On the other hand, it seems that palladium, when bound in an organic compound (linked to TEA in Pd complex), does not contribute significantly to cardio-toxicity in our experimental conditions.

Keywords: Palladium, cardio-toxicity, isolated heart.

INTRODUCTION

Palladium (Pd) is a heavy metal belonging to the platinum group of elements. It is obtained as a by-product during the extraction of platinum from the residue of copper and nickel refineries, but little is known about its toxicity. Previous studies have shown that palladium when administered intravenously in the form of inorganic compounds ($Pd(NO_3)_2$, $PdCl_2$, $(NH_4)_2PdCl_4$, K_2PdCl_4 and PdSO4) may cause arrhythmias and decrease in blood pressure of an unanesthetized rat [1]. This effect is associated with changes of cardiomyocyte membrane potential [1]. Significant toxicity of palladium in rat kidney was also described: it inhibits both the Na(+)-Ca(2+)-antiporter and the Na(+)-H(+)-exchanger in tubulocytes, leading to retention of sodium and calcium [2]. However, palladium is not genotoxic in mammalian and bacterial cells [3]. In humans, palladium may cause allergy, and cross-reacts with nickel [4], but other specific toxicities except mucous irritation [5, 6] were not described.

On the other hand, there are some indications that palladium in the form of organic compounds is not toxic, but may have antioxidant and cardioprotective effects [7, 8]. Alpha-lipoic acid is well known as a powerful biological antioxidant and its therapeutic potential has been explored extensively [9]. However, it was shown that the complex of palladium and alpha-lipoic acid is five times more potent antioxidant than the alpha-lipoic acid itself [8].

The aim of our study was to evaluate and compare toxicity of inorganic and organic palladium compounds on the isolated rat heart.

MATERIALS AND METHODS

Isolated Rat Heart Preparation

The hearts (total number *n*=30, 6 for each experimental group; discarded hearts were not included in total number of

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hearts) excised from Wistar albino rats, male sex, age 8 weeks, body mass 180-200 g (obtained from Military Medical Academy, Belgrade, Serbia) were perfused with Langendorff apparatus (Experimetria Ltd, 1062 Budapest, Hungary). After short-term ether narcosis, animals were killed by cervical dislocation (Schedule 1 of the Animals/Scientific Procedures, Act 1986, UK). After urgent thoracotomy and rapid heart arrest by superfusion with ice-cold isotonic saline, the hearts were rapidly excised, isolated, the aortas were cannulated and retrograde perfused according to the technique for the condition of constant perfusion pressure (CPP). The composition of the non-recirculating Krebs-Henseleit perfusate was as follows (mmol/1): NaCl 118, KCI 4.7, $CaCI_2 \times 2H_2O$ 2.5, $MgSO_4 \times 7H_2O$ 1.7, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 5.5, equilibrated with 95% O_2 plus 5% CO_2 and warmed to 37°C (pH 7.4).

Immediately after the establishment of automatic operation, through the created opening in the left atrium of the heart, and destroyed mitral valve, the sensor was inserted (transducer BS4 73-0184, Experimetria LTD, Budapest, Hungary) in the left ventricle to register the pressures.

Physiological Assay and Experimental Protocol

All study groups underwent 30-min stabilization perfusion at CPP of 70 cmH_2O. In order to test coronary vascular reactivity, all hearts were challenged by short-term occlusions (5-30 s) as well as by bolus injection of 5 mmol/1 adenosine (60 µl at a flow rate of 10 ml/min in order to elicit maximal CF) during the stabilization period. The hearts were discarded (about 25%) if the flow did not increase by 100% over the control value (for both tests). Properly performed control experiments were included in the study (i.e., the groups of the hearts in which the CPP/CF relationship is studied twice in the absence of any drug for 120 minutes). It was essential to confirm that the used preparation was stable and that the responses to the first and the second run of changes in perfusion pressure did not differ substantially, as we described previously [10].

Experimental group in which we investigated effects of different palladium compounds, hearts were perfused with increasing concentrations of:

1. triethanolamine (TEA) (67 nM/l, 670 nM/l, 6700 nM/l, 67000 nM/l and 670000 nM/l)
2. triethanolamine acetate (TEAA) (48 nM/l, 480 nM/l, 4800 nM/l, 48000 nM/l and 480000 nM/l)
3. palladium(II) chloride ($PdCl_2$) (56 nM/l , 560 nM/l , 5600 nM/l , 56000 nM/l and 560000 nM/l) and
4. *trans*-dichlorobis(triethanolamine-*N*)palladium(II) complex (*trans*-[$PdCl_2(TEA)_2$]) (21 nM/l, 210 nM/l, 2100 nM/l, 21000 nM/l and 210000 nM/l).

After stabilization period of 30 minutes ("zero" minute), perfusion with an increasing concentration of each compound that started every 30 minutes (30, 60, 90, 120 minute). The flow was considered as stable at each value of perfusion pressure, when 3 repeated values of CF were the same.

In control and experimental group, the following parameters of heart function were continuously registered:

1. maximum rate of left ventricular pressure development (dP/dt max)
2. systolic left ventricular pressure (SLVP)
3. diastolic left ventricular pressure (DLVP)
4. mean left ventricular pressure (MBP) and
5. heart rate (HR).

Substances

Triethanolamine acetate and *trans*-dichlorobis (triethanolamine-*N*)palladium(II) complex were orginal products of the research laboratory at Department of Chemistry, Faculty of Sciences, University of Kragujevac, Serbia (Fig. **4**). Triethanolamine and palladium(II) chloride were purchased from the company Merck (Darmstadt, Germany).

Statistical Analysis

The concentration-response relationship was determined by linear regression on logarithmically transformed data calculated according to the method of least squares. The effects of various concentrations of tested substances were expressed as percentage of the maximal response. The range of values used for the linear regression was from 15% to 85% of the maximal response, in the more linear part of the curve. The concentration of an agonist eliciting 50% of the maximum response (EC_{50}) and its confidence limits (1,96 x standard error) were determined graphically for each concentration-response curve by linear interpolation [11]. Significance of the linear regression was tested by analysis of variance, with maximal acceptable probability of null hypothesis being 0.05.

RESULTS

Maximum Rate of Left Ventricular Pressure Development (dP/dt max)

The maximum rate of left ventricular pressure development (dP/dt max) was not affected significantly by either TEAA (from 4.8 x 10^{-8}M to 4.8 x 10^{-4}M; F=1.15, df_1 = 4, df_2 = 20, p > 0,05), TEA (from 6.7 x 10^{-8}M to 6.7 x 10^{-4}M; F=1.07, df_1 = 4, df_2 = 20, p > 0,05), $PdCl_2$ (from 5.6 x 10^{-8}M to 5.6 x 10^{-4}M; F=2.17, df_1 = 4, df_2 = 20, p > 0,05), or Pd complex (from 2.1 x 10^{-8}M to 2.1 x 10^{-4}M; F=0.53, df_1 = 4, df_2 = 20, p > 0,05).

Systolic Blood Pressure in the Left Ventricle (SLVP)

The systolic blood pressure in the left ventricle (SLVP) was not affected significantly by either TEAA (from 4.8 x 10^{-8}M to 4.8 x 10^{-4}M; F = 1.59, df_1 = 4, df_2 = 20, p > 0,05), TEA (from 6.7 x 10^{-8}M to 6.7 x 10^{-4}M; F = 3.84, df_1 = 4, df_2 = 20, p > 0,05), $PdCl_2$ (from 5.6 x 10^{-8}M to 5.6 x 10^{-4}M; F = 1.33, df_1 = 4, df_2 = 20, p > 0,05), or Pd complex (from 2.1 x 10^{-8}M to 2.1 x 10^{-4}M; F = 1.02, df_1 = 4, df_2 = 20, p > 0,05).

Diastolic Blood Pressure in the Left Ventricle (DLVP)

The diastolic blood pressure in the left ventricle (SLVP) was significantly decreased by both TEAA (maximal decrease from baseline value 10 ± 5%; from 4.8 x 10^{-8}M to

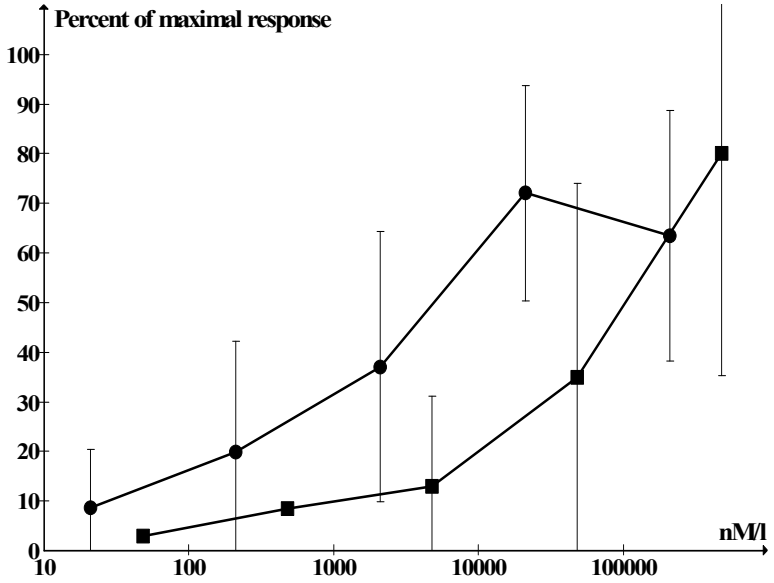

Fig. (1). Decrease of diastolic blood pressure in the left ventricle of the isolated rat heart caused by $PdCl_2$ (●) and TEAA (■). Each point represents mean response of isolated heart taken from 6 different animals. Error bars = standard deviations.

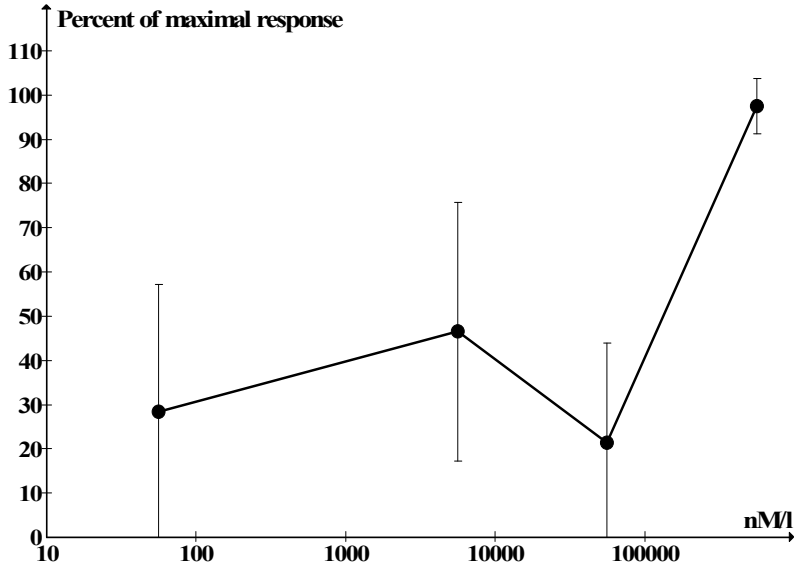

Fig. (2). Decrease of mean blood pressure in the left ventricle of isolated rat heart caused by $PdCl_2$ (●). Each point represents mean response of isolated heart taken from 6 different animals. Error bars = standard deviations.

4.8 x 10^{-4}M; F = 4.20, $df_1 = 4$, $df_2 = 20$, $p < 0,05$; EC50 = 2.9 ± 0.01 x 10^{-5}M) and $PdCl_2$ (maximal decrease from baseline value 12 ± 6%; from 5.6 x 10^{-8}M to 5.6 x 10^{-4}M; F = 4.61, $df_1 = 4$, $df_2 = 20$, $p < 0,05$; EC50 = 2.6 ± 0.01 x 10^{-5}M) (Fig. **1**), while TEA (from 6.7 x 10^{-8}M to 6.7 x 10^{-4}M; F = 0.85, $df_1 = 4$, $df_2 = 20$, $p > 0,05$) and Pd complex (from 2.1 x 10^{-8}M to 2.1 x 10^{-4}M; F = 0.48, $df_1 = 4$, $df_2 = 20$, $p > 0,05$) did not show significant effect.

Mean Blood Pressure in the Left Ventricle (MBP)

The mean blood pressure in the left ventricle (MBP) was significantly decreased only by $PdCl_2$ (maximal decrease from baseline value 15 ± 8%; from 5.6 x 10^{-8}M to 5.6 x 10^{-4}M; F = 10.62, $df_1 = 4$, $df_2 = 20$, $p < 0,01$; EC50 = 2.8 ± 0.02 x 10^{-5}M) (Fig. **2**), while TEAA (from 4.8 x 10^{-8}M to 4.8 x 10^{-4}M; F = 3.30, $df_1 = 4$, $df_2 = 20$, $p > 0,05$), TEA (from 6.7 x 10^{-8}M to 6.7 x 10^{-4}M; F = 3.30, $df_1 = 4$, $df_2 = 20$, $p > 0,05$) and Pd complex (from 2.1 x 10^{-8}M to 2.1 x 10^{-4}M; F = 2.40, $df_1 = 4$, $df_2 = 20$, $p > 0,05$) did not show significant effect.

Heart Rate (HR)

The isolated heart rate (HR) was significantly decreased by $PdCl_2$ (maximal decrease from baseline value 26 ± 16%; from 5.6 x 10^{-8}M to 5.6 x 10^{-4}M; F = 4.15, $df_1 = 4$, $df_2 = 20$, $p < 0,05$; EC50 = 2.4 ± 0.1 x 10^{-6}M), TEAA (maximal decrease from baseline value 34 ± 18%; from 4.8 x 10^{-8}M to 4.8 x 10^{-4}M; F = 11.32, $df_1 = 4$, $df_2 = 20$, $p < 0,01$; EC50 = 1.3 ± 0.1 x 10^{-5}M), TEA (maximal decrease from baseline value 5 ± 4%; from 6.7 x 10^{-8}M to 6.7 x 10^{-4}M; F = 4.24, $df_1 = 4$, $df_2 = 20$, $p < 0,05$; EC50 = 1.7 ± 0.01 x 10^{-5}M) and Pd complex (maximal decrease from baseline value 6 ± 5%;

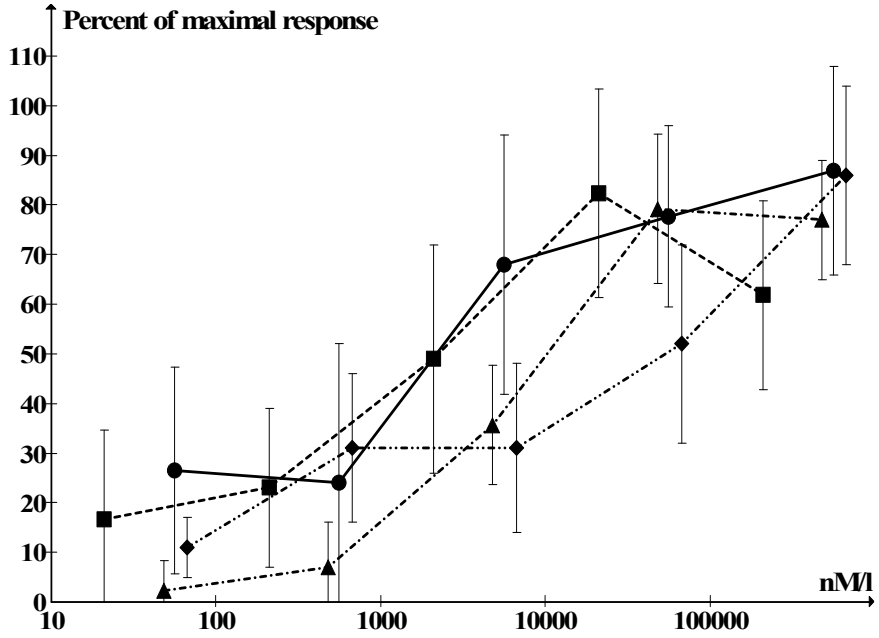

Fig. (3). Decrease of the isolated rat heart rate caused by $PdCl_2$ (●), Pd complex (■), TEAA (▲) and TEA (◆). Each point represents mean response of isolated heart taken from 6 different animals. Error bars = standard deviations.

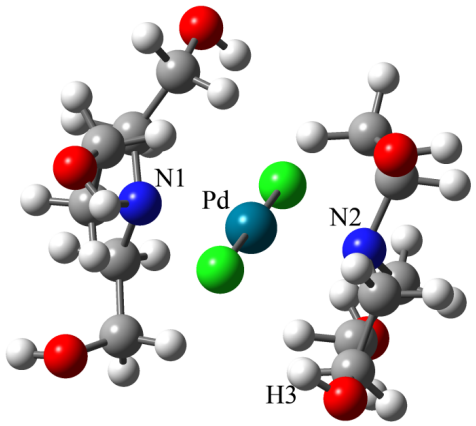

Fig. (4). The optimized geometry of *trans*-$[PdCl_2(TEA)_2]$ complex.

from 2.1 x 10^{-8}M to 2.1 x 10^{-4}M; F = 2.91, $df_1 = 4$, $df_2 = 20$, $p < 0,05$; EC50 = 3.5 ± 0.1 x 10^{-6}M) (Fig. **3**).

DISCUSSION

Published data on effects of palladium on heart are limited to just two in vivo studies on rabbits and rats, where after intravenous administration of palladium chloride a drop in blood pressure was noted, and some of the experimental animals died [1]. However, except observation that palladium causes profound "disturbance of the electrical integrity of the ventricular myocardium" [12], no other explanation about possible mechanism of toxicity was offered. In our study, we have noted clear depression of the isolated rat heart contractility caused by inorganic palladium compound (palladium(II) chloride), manifested as drop in diastolic and mean pressure in the left ventricle, and as decrease of the heart rate. Other investigated substances did not show consistent effects, and their inhibitory effect was limited to the heart rate. Since TEA and its Pd(II) complex decreased the heart rate to small extent, and palladium is linked to TEA in Pd complex, it seems that palladium, when bound in an organic compound, does not contribute significantly to cardiotoxicity. Therefore, inorganic compounds of palladium are more toxic to myocardium than organic ones, which may have certain implications for the treatment of patients poisoned with palladium.

Palladium ions may inhibit majority of intracellular enzymes, while its metallic form is not cytotoxic [13-15]. In similar concentrations as used in our experiments (micromolar range) palladium(II) chloride blocked DNS synthesis in vivo in rats, and inhibited functioning of creatin kinase, an important enzyme of energy metabolism [16]. This cytotoxic effect of palladium ion could explain for depressant effect of palladium chloride on isolated rat heart, observed in our study.

It is interesting that acute and chronic toxicity studies in rodents and rabbits with palladium compounds did not show histopathological effects in myocardium, suggesting subtle and specific effect of palladium on heart [16-18]. It seems that palladium ions interfere with binding of calcium, magnesium and other bi-valent ions to ion channels and enzymes [19-22], leading to disturbances in membrane potential, decreased entry of calcium in cells, decreased contractility and arrhythmias.

Reports on toxicity of palladium in humans are limited to studies on skin allergy caused by palladium [23], but systemic toxic effects could not be excluded. Although bioavailability of palladium after oral administration is only 0.5%, it may be absorbed by inhalation and through inflamed skin in quantities large enough to cause systemic toxic effects [23]. Also, it is interesting that 'till now is not investigated toxic effects of palladium *in vivo*. Cardiac toxicity observed on isolated rat heart in our study should inspire further research on toxicity of palladium investigated *in vivo* on

different experimental models, as well as, in human organism.

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