ANTI-ARRHYTHMIC PROPERTIES OF DADLE IN OUABAIN-INDUCED ARRHYTHMIA IN RATS

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Abstract - Opioid receptors open the potassium channels and block the voltage-gated calcium channels and result in membrane hyperpolarization. Hence, they possess negative inotropic effect on the heart function. The cardiac glycoside, ouabain depolarizes the cell membrane that leads to ouabain-induced arrhythmia. The aim of the present experiment was to evaluate the antiarrhythmic properties of the specific delta opioid receptor agonist, D-Ala, D-Leu Enkephalin (DADLE) on ouabain-induced arrhythmia in isolated rat atria. Eighteen male rats including the control and DADLE-incubated (100 nM or 1 µM) ouabain-stimulated groups were used. After induction of anesthesia, the atria were isolated and spontaneously beating rate, chronotropic responsiveness, and onset of arrhythmia and asystole were assessed following incubation by DADLE and stimulation by ouabain using standard organ bath. DADLE significantly postponed the onset of arrhythmia compared to control group ($p < 0.01$). Although it did not delay the onset of asystole, but was able to significantly increase the heart rate in isolated rat atria ($p < 0.01$). Moreover, it diminished the positive chronotropic effect of ouabain ($p < 0.05$). Our results revealed that DADLE could diminish the ouabain-induced arrhythmia in isolated rat atria. The hyperpolarization of cell membrane or reduction of ouabain toxicity might mediate this beneficial effect, which needs for further experiments.

Index terms - DADLE, Opioids, Cardiovascular, Arrhythmia, Atria

I. INTRODUCTION

Research has demonstrated that morphine can significantly decrease the incidence of coronary artery occlusion-induced ischemic ventricular arrhythmia. Also, it has been reported that long-term treatment of rats with high doses of morphine markedly alters the function of myocardial G-protein-regulated adenylyl cyclase signaling. These changes are associated with a reduced susceptibility to ischemia-induced ventricular arrhythmias [1]. Previous research has indicated that the opioid-receptors, especially δ-opioid receptors, mediate cardioprotection [2]. Delta (δ)- and kappa (κ)-opioid receptors stimulation protects cardiomyocytes from ischemic injury decreases apoptosis. Additionally, it is reported that ouabain- induced arrhythmia displays inhibition of Na^+/K^+ ATPase pump that is accompanied to cell membrane depolarization that leads to ouabain-induced arrhythmia [3].

Moreover, there are ouabain-induced cellular toxicity that is related to intervention in signal transduction and Ras-Ros pathway that leads to ouabain-induced cell death [4]. However, to date, there have been no therapies that reported to treat arrhythmia without any adverse effects. Given the protective effects of opioids, it is possible for them to be therapeutically utilized in atrial arrhythmia, specifically in cardiac glycoside-induced arrhythmia. As such, the present study was designed to explore such possibility, particularly, the antiarrhythmic activity of D-Ala, D-Leu Enkephalin (DADLE) -a specific δ-opioid receptor agonist- on ouabain-induced arrhythmia in rat atria and identifies the underlying mechanisms of such action.

II. MATERIAL AND METHODS

Male Wistar albino rats (body weight 250-280g) were obtained from the Department of Pharmacology Comparative Biology Unit (Tehran University of Medical Sciences). All animal procedures were in accordance with 'Guide for the Care and Use of Laboratory Animals' (NIH US publication No 85-23, revised 1985) recommendations. The animals (n=18) were randomly divided into three groups, namely control ouabain-stimulated group and two different ouabain-stimulated groups incubated with different concentration of DADLE (100 nM or 1 µM). Isolated spontaneously beating atria were used to study cardiac chronotropic response to ouabain and DADLE treatment. General anesthesia was induced by an intraperitoneal injection of a combination of ketamine and diazepam (100 mg/kg, 2 mg/ kg, respectively).

The hearts were then excised and used for in vitro study. The rat atria were isolated in cold oxygenated physiological salt solution. The whole atria were used for recording the spontaneous atrial beating and atrial arrhythmias using the PowerLab system (ADInstrument, Australia) [5]. Statistical analyses were carried out using GraphPad Prism 5 software (San Diego, CA, USA). All results were expressed in mean ± SEM. One-way analysis of variance (ANOVA) was used for the evaluation of the effect of two different doses of DADLE. Dunnett's multiple comparison test was then performed for multiple

comparisons. P-values less than 0.05 were considered as statistically significant.

III. RESULTS

The patterns of spontaneous contractions of isolated rat atria, ouabain-induced arrhythmia, and ouabain-induced asystole were shown in Figure 1. Ouabain alone (230 µM) produced arrhythmia after 1.22 ± 0.08 min and asystole after 2.5 ± 0.27 min. DADLE was not arrhythmogenic in all of the treated groups since it was evaluated for 30 min after the onset of treatment. Subsequently, the onset of arrhythmia and asystole following ouabain stimulation in DADLE-treated groups, was calculated. It was observed that the time of onset of arrhythmia was significantly delayed in DADLE-treated groups compared to the control group ($p < 0.01$, Figure 2).

These times were 2.26 ± 0.08 min and 2.11 ± 0.19 min for DADLE in concentrations of 1 µM and 100 nM, respectively. However, DADLE could not statistically change the onset of asystole in all DADLE-treated groups ($p > 0.05$, Figure 2). DADLE (100 nM) could significantly increase the atrial beating rate compared to the control group (370 ± 6.99 beats/min, and 290 ± 1.21 beats/min, respectively, $p < 0.01$, Figure 3). Although ouabain increased the atrial beating rate significantly in control group ($p < 0.05$), but it could not boost the atrial beating rate in DADLE-treated groups (Figure 3).

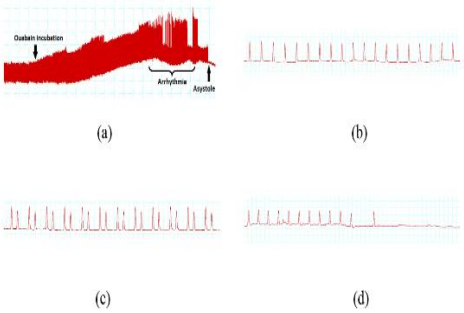


Figure 1: The pattern of contractions of isolated rat atria after ouabain stimulation. A complete record of isolated atrial beats (a), atrial beatings before incubation of ouabain (b), ouabain-induced arrhythmia (c), and ouabain-induced asystole (d).

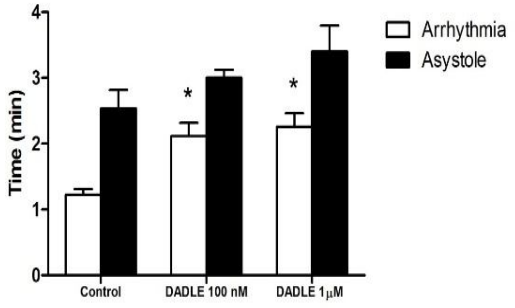


Figure 2: Time of onset of arrhythmia and asystole after ouabain incubation in control and treatment groups. Data are shown as mean ± SEM. Six rats were used in each group. *p < 0.01 compared to time of arrhythmia in control group.

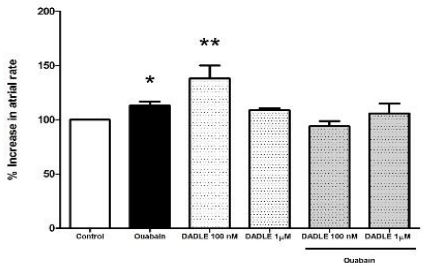


Figure 3: Atrial beating rates in different groups. Data are shown as mean ± SEM. Six rats were used in each group. *p < 0.05, **p < 0.01 compared to control group.

DISCUSSION

Opioid receptor stimulation causes the closure of voltage-gated Ca^{2+} channels resulting in the activation of K^+ channels thereby leading to the hyperpolarization of myocyte cell membrane [6]. It is reported that the δ-agonists, methionine enkephalin and leucine encephalin, have an effect on phosphatidylinositol pathway promote Ca^{2+} depletion of reticulum sarcoplasmic that induce negative inotropic effect [3]. Moreover, it is recently reported that enkephalin derivatives effectively bind to the µ- and δ-opioid receptors and are involved in cardioprotective mechanism in rat heart and have therapeutic potential for the treatment of ischemic heart disease through a nitric oxide (NO)-K_{ATP}-mediated mechanism [7]. Also, it is suggested that cardiac glycosides secondary to an interaction with (but not inhibition of) Na^+/K^+-ATPase (NKA) pump induce intracellular signaling through Src kinase. It leads to reactive oxygen species (ROS) production and mitochondrial ATP-dependent K^+ channels (mito-K_{ATP}) opening [8]. With regard to these findings that show opioid agonists can, in addition to influencing the heart inotropic function, have protective role in heart, in the present study we focused on DADLE-the specific agonist of δ-opioid receptor- and investigated its potential therapeutic role in preventing or delaying the ouabain-induced arrhythmia. It is reported that both, the therapeutic and toxic effects of ouabain are the result of Ca^{2+} influx through cardiac myocytes [9]. Besides, cardiac glycoside toxicity induces altered function of cardiac ryanodine receptors (RyRs) through reactive oxygen species (ROS) which leads to spontaneous Ca^{2+} waves. ROS generation oxidizes thiol groups on RyRs, which increases their functional activity through increasing sensitivity to Ca^{2+} in the sarcoplasmic reticulum [10]. It was reported that there is not a direct relationship between membrane depolarization because of ouabain related Na^+/K^+ ATPase pump inhibition and ouabain-induced cellular toxicity. It seems that its toxicity is related to intervening in signal transduction and Ras-Ros pathway as well as the glutathione that plays a major role in ouabain-induced cell death [4]. Moreover, δ_2-opioid receptor plays a central role in protecting cardiomyocytes from apoptosis during injury [11].

Ras is a well-known modulator of apoptosis and suppression of protein kinase C (PKC) activity. It specifically induces apoptosis through constitutive activated Ras protein [12]. Our data showed DADLE in concentration of 100 nM and 1 µM delays the onset of arrhythmia approximately two-folded in ouabain-stimulated isolated rat atria but its underlying mechanism was not investigated in the present study. Nevertheless, it may be due to membrane hyperpolarization or improved viability and reduced apoptosis via suppression of Ros-Ras pathway that arises in response to activation of PKC. Reports indicate that δ- and κ-opioid receptor stimulation by δ-agonists, methionine enkephalin and leucine enkephalin (100 pM to 1 µM), has an effect on phosphatidylinositol pathway that promote Ca^{2+} depletion of reticulum sarcoplasmic which induces negative inotropic effect. Also, DADLE protected the heart against preconditioning ischemia in concentration of 10 nM, but it had less beneficial in higher dose of 1 µM [3]. The role of reticulum sarcoplasmic Ca^{2+} ATPase pump has been evaluated in cardiprotective mechanism of κ- and δ-opioid receptors in ischemia/reperfusion injury in the rat heart. It was seen that both of them caused two time decrease in creatine phosphokinase release [13]. DADLE is a K^{+} channel opener, which may delay ouabain-induced arrhythmia through closing voltage-gated Ca^{2+} channels which subsequently causes the K^{+} channels to open and the cell membrane to hyperpolarize. However, δ-opioid receptor activation can decrease ouabain-induced myocyte injury because it is seen that DADLE has an oxygen radical scavenger role in heart since ouabain promote cell death by oxygen radical induction in cells. Therefore, it may have major role in protecting myocyte against ouabain-induced arrhythmia. It was reported that δ-opioid-mediated cardioprotection have the potential to change the clinical pharmacology in terms of prevention and treatment of life-threatening conditions like myocardial infarction [2]. In this study, we also evaluated the heart rate in under investigated groups. It has been already shown that δ- and κ-opioid receptors do not play a role in basal or sympathetic stimulated heart rate [13]. Our data indicated that while ouabain and DADLE, separately, induced significant tachycardia, ouabain failed to increase heart rate in DADLE-treated groups. Following the evaluation of the effect of these two drugs on the heart rate, it seems that there is an interference between ouabain and DADLE signaling on isolated beating atria.

CONCLUSION

It is concluded that DADLE shows antiarrhythmic properties in reducing the cardiac arrhythmias. DADLE opens the potassium channels, which may correct the ouabain-induced ionic disturbances. Moreover, this effect might be due to a reduction of ouabain toxicity in myocardium through improving the myocardial viability and reducing the apoptosis.

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