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Antiarrhythmic action of creatine in acute myocardial

Acción antiarrítmica de la creatina en el infarto agudo del miocardio

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RESUMEN

Introducción A pesar de su degradación por ectonucleotidasas, la concentración intersticial del trifosfato de adenosina ($[ATP]_{out}$) aumenta después de un infarto agudo del miocardio (IAM) y se correlaciona con la ocurrencia de latidos ventriculares prematuros y taquicardia ventricular. Recientemente se demostró que el ATP extracelular activa una corriente despolarizante arritmogénica transportada por canales TRP ("Transient Receptor Potential") TRPC3/7. Una estrategia para prevenir las arritmias durante un episodio isquémico agudo puede ser reducir $[ATP]_{out}$. Resultados previos de nuestros laboratorios, en un modelo de IMA en rata, sugieren que este puede ser el caso.

Objetivos Estudiar si la creatina extracelular puede servir de "buffer" para el incremento súbito de $[ATP]_{out}$ y reducir los eventos arrítmicos en un modelo de IMA en corazón aislado de rata.

Método Se registraron las actividades eléctrica y contráctil en corazones aislados de rata perfundidos con la técnica de Langendorff. La arteria coronaria izquierda se ligó para inducir un infarto del miocardio.

Resultados En condición control, la creatina (1-3 mmol/L) no tuvo efectos importantes sobre las duraciones del QRS y del QT, así como tampoco sobre el intervalo RR o la fuerza de contracción. La ligadura de la arteria coronaria izquierda redujo marcadamente el umbral de fibrilación ventricular (UFV). No obstante, cuando los corazones fueron tratados previamente con creatina (1-3 mmol/L), el UFV fue significativamente mayor después del IMA, cuando se comparó con los corazones no tratados. Más aún, en corazones perfundidos con creatina después de ligar la arteria coronaria, el UFV también se incrementó pero en menor magnitud.

Conclusión Nuestros resultados sugieren que la creatina, a una concentración relativamente alta, puede servir como "buffer" para la liberación súbita de ATP durante la fase inicial de la isquemia y tener, por tanto, un efecto antiarrítmico.

Palabras clave: ATP, creatina, corazón, infarto del miocardio, arritmias.

ABSTRACT

Introduction Despite its degradation by ectonucleotidases, interstitial adenosine triphosphate concentration ($[ATP]_{out}$) increases after an acute myocardial infarction (AMI) and correlates with the occurrence of ventricular premature beats and ventricular tachycardia. It has been recently shown that extracellular ATP activates a depolarizing arrhythmogenic current carried by the transient receptor potential (TRP) channels TRPC3/7. A decrease in $[ATP]_{out}$ could be a strategy to prevent arrhythmias during an acute ischemic episode. Previous results from our laboratories in a rat model of AMI suggest that this could be the case.

Objective To study whether extracellular creatine would serve as a buffer for the sudden increase of $[ATP]_{out}$ and decrease arrhythmic events in an isolated rat heart model of AMI.

Method Electrical and contractile activities were recorded on isolated Langendorff perfused rat hearts. The left coronary artery was occluded to induce a myocardial infarction.

Results Under control condition, creatine (1-3 mmol/L) had no major effects on QRS and QT durations nor on RR interval or force of contraction. The ligature of the left coronary artery markedly decreased the ventricular fibrillation threshold (VFT). However, when hearts were previously treated with creatine (1-3 mmol/L) the VFT was significantly higher after the AMI compared to untreated hearts. Moreover, in hearts perfused with creatine after coronary ligature, VFT was also increased but to a lesser extent.

Conclusion Our results suggest that creatine, at a relatively high concentration, would serve as a buffer for the sudden release of ATP during the early phase of ischemia thus having an antiarrhythmic effect.

Key words: ATP, creatine, heart, myocardial infarction, arrhythmias.

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INTRODUCTION

Besides its role in cellular energetics and metabolism adenosine triphosphate (ATP), can modulate the activity of several tissues including the heart. [1] In normal conditions, due to continuous degradation by ecto-ATPases, extracellular ATP concentration is in the nanomolar range. However, under pathological situations such as myocardial ischemia and infarction, more specifically during the initial phase of an acute myocardial infarction (AMI), the extracellular (interstitial) ATP concentration ($[ATP]_{out}$) could rise locally to a micromolar level [2, 3] or even hundreds of micromoles during thrombus formation due to an impaired blood flow.[4] The increase in $[ATP]_{out}$ (and also of uridine triphosphate, UTP) during the early phase of an AMI triggers a cationic (nonspecific) current that induces abnormal automatic activity and arrhythmias.[5] Alvarez and co-workers [6], demonstra-ted that a sudden increase in extracellular ATP (such as it occurs during a myocardial

infarction or ischemia) activates a cationic non-specific current that is transported through the heteromeric transient receptor potential TRPC3/C7 channel. The signaling cascade that leads to TRP channel activation includes: the activation of a P2Y2 purinergic receptor by free ATP (ATP^{-4}), activation of a G_q protein with the subsequent activation of the β isoform of phospholipase C ($PLC_{□}$) which in turn hydrolyzes the phosphatidyl inositol 4, 5-bisphosphate into diacyl glycerol (DAG) and inositol-1, 4, 5-triphosphate (IP_3). DAG activates the protein kinase C (PKC) which phosphorylates and activates the TRPC3/C7 channel.[6] TRP channels are expressed in almost all mammalian cells including human and are responsible of the under-lying mechanisms of important physiological functions and physiopathological conditions.[7, 8]

From a physiological point of view elucidation of this basic mechanism of ATP-induced arrhythmias is important but it is also relevant to therapeutics since one or more new pharmacological targets were defined. This could allow the development of compounds acting on these targets to prevent cardiac arrhythmias. In this sense, results from our laboratories indicated that in a rat model creatine could prevent arrhythmias during the early phase of an AMI.[9] The rationale for this is as follows: The existence of ecto-nucleoside diphosphate kinases (NDPK) has long been documented.[10] The NDPK transfers phosphate groups between ATP and creatine to form creatine phosphate or vice versa. Thus, an excess creatine could prevent the action of ATP^{-4}, which will be degraded to ADP (inactive on the TRPC3/C7 channel) to form creatine phosphate by the NDPK. Creatine is naturally synthesized in liver and kidneys from the amino acids L-arginine, glycine and L-methionine and is transported to and used in the energy metabolism in muscles.[11] Creatine is widely used as a natural dietetic supplement and by high performance athletes.[12]

The aim of the present work was to extend our studies to an isolated rat heart model and to determine whether creatine could have an antiarrhythmic action during an AMI.

METHODS

Experiments were performed on male adult Wistar rats. Under penthobarbital anesthesia, hearts were carefully dissected and mounted in a Langendorff column. They were perfused at constant flow (10 mL/min) with a Tyrode solution of the following composition (mmol/L): NaCl 140; KCl 2.5; $MgCl_2$ 0.5; $CaCl_2$ 2; Tris-hydroxymethylaminomethane 10; Glucose 5 (pH = 7.4, gassed with O_2; T = 35°C). Creatine (Sigma, USA) was directly dissolved in Tyrode solution. A bipolar platinum recording electrode was placed on the right ventricular epicardium and the surface electrocardiogram (ECG) was recorded with a biophysical amplifier (AVB-10; Nihon Kohden, Japan). Another bipolar platinum electrode was placed near the atrioventricular ring and was connected to an electronic stimulator (SEN-7103; Nihon Kohden, Japan) through a stimulus-isolating unit (SS-102J; Nihon Kohden, Japan). Cardiac apex was fixed to a force-displacement transducer (SB-1T; Nihon Kohden, Japan) with a surgical 5-0 silk thread. The force of contraction (FC) was recorded using a low-level DC preamplifier (ACH-8; Nihon Kohden, Japan). Analog signals (ECG and FC) were digitized (100 μs sampling interval) using an AD/DA converter (Labmaster TL-1-125, Mentor, Ohio, USA), stored on a PC and analyzed off-line with the ACQUIS1 software (CNRS License, France). ECG and FC values were recorded at the spontaneous heart rate. Hearts were let to stabilize for at least 30 min before beginning the experiments. The ventricular fibrillation threshold (VFT) was determined using a stimulation program that consisted of twenty 2-ms duration current pulses at 2-ms interval. Current intensity (mA) was measured with the stimulus-isolating unit. VFT was the current intensity at which at least 5 spontaneous arrhythmic complexes were observed after the end of the stimulus train. All recordings were made under control condition and after occlusion of the left coronary artery at pro-ximal point below the left atrial appendage using a surgical 0 silk thread.

Three experimental series were performed. In the first one (N = 6), hearts were perfused under control conditions and after a 30 min stabilization period, the physiological variables were recorded. Thereafter, the left coronary artery was occluded and after 5 min all physiological variables were again recorded. In the second experimental series (N = 6), hearts were initially perfused with control solution and after all physiological variables were recorded, creatine (1 or 3 mmol/L) was added to the Tyrode solution. Hearts were let to stabilize for 10 min and all physiological variables were again recorded. After this, and always in the presence of creatine, the left coronary artery was occluded and the physiological variables were recorded as in the first experimental series. The third experimental series (N = 5) was the same as the first one except that after left coronary artery occlusion, creatine (1 or 3 mmol/L) was added to the perfusion solution.

Tabla 1. Electrical and contractile activities of spontaneous isolated rat hearts in control condition and after an Acute Myocardial Infarction (AMI): Absence of effects of creatine (1 - 3 mmol/L)

	RR (ms)	QRS duration (ms)	QTc (ms)	Force of Contraction (mN)	Contraction duration (ms)
Control	285.0 ± 16.5	13.7 ± 2.5	11.9 ± 0.7	154.3 ± 12.6	223.8 ± 10.1
AMI	312.6 ± 27.9 *	13.5 ± 2.3	12.1 ± 1.5	101.0 ± 20 *	242 ± 12.2
Creatine before AMI	305.8 ± 28.8	12.0 ± 2.8	12.1 ± 1.5	149.9 ± 5.2	227.0 ± 17.6
Creatine + AMI	365.9 ± 39.9 *	15.8 ± 1.7	12.1 ± 0.8	82.7 ± 20.1 *	232.5 ± 19.7
Creatine after AMI	368.7 ± 15.3 *	15.3 ± 3.9	9.5 ± 0.8	83.9 ± 14.0 *	260.2 ± 24.9

Data for AMI (with or without creatine) represent values obtained after 5 minutes of coronary artery occlusion. * $p < 0.05$, with respect to control. $QT_c = QT/\sqrt{RR}$

Results were analyzed using a Student's t-test for paired or unpaired samples as required using the Gnumeric Spreadsheet (Gnome Project, version 1.10.17). Differences were considered statis tically significant for $p < 0.05$. Results are expressed as Means ± Standard Errors of Means.

RESULTS

Table 1 summarizes values for RR interval, QRS duration, corrected QT duration ($QT_C = QT/\sqrt{RR}$) and FC under control condition. In this condition the VFT was 42.0 ± 3.2 mA (N = 17). Since results obtained with creatine 1 or 3 mmol/L were not statistically different, they were pooled together and are presented as "creatine". As can be seen in Table 1, under control condition creatine had no statistically significant effects on the electrical and contractile activities or on the VFT. There was a tendency, however, to increase the RR interval, which was not statistically significant. Acute myocardial infarction (AMI) was achieved by occluding the left coronary artery (see Methods). Successful occlusion was recognized by pallor of the anterior left ventricular wall and by the occurrence of immediate decrease in contractile force. Acute myocardial infarction (5 minutes), induced by left coronary artery occlusion (AMI), significantly ($p < 0.05$) increased RR interval and decreased the force of contraction FC by 28 ms and 35%, respectively (Table 1). At this time, the VFT was significantly decreased by 62.1 ± 12.6 % ($p < 0.05$; Figure 1).

Creatine did not prevent the increase in RR interval or the decrease in FC by myocardial infarction (Table 1). However, in creatine-treated hearts after an AMI the VFT was significantly higher than in control infarcted hearts (Figure 1). Moreover, although creatine treatment after AMI did not recover RR or FC values (Table 1) it exhibited a tendency to increase the VFT (Figure 1).

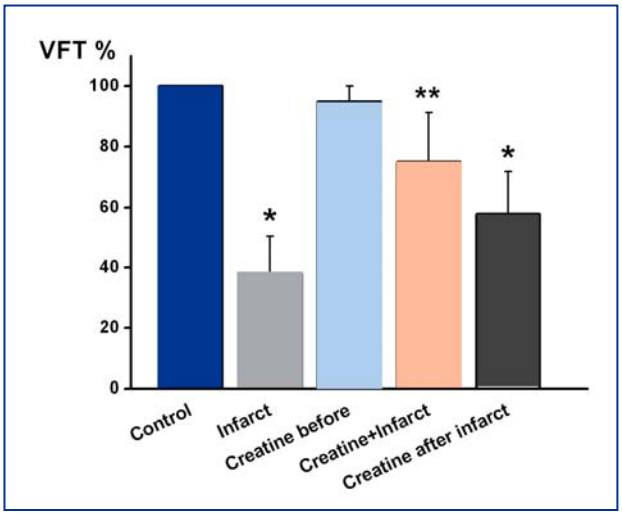

Figure 1: Creatine increased the ventricular fibrillation threshold (VFT) during an acute myocardial infarction (AMI) in isolated Langendorff perfused rat hearts. Ligature of left coronary artery markedly decreased the ventricular fibrillation threshold (VFT). However, when hearts were previously treated with creatine (1 - 3 mmol/L) VFT after the AMI was significantly higher than in untreated hearts. Moreover, in hearts perfused with creatine after coronary ligature, VFT was also increased but to a lesser extent. * $p < 0.05$ with respect to control; ** $p < 0.05$ with respect to infarct.

DISCUSSION

The present study strengthens the idea that creatine could have an antiarrhythmic action in the early stages of an acute myocardial infarction, extending and confirming our previous results in a rat model of AMI 9. In that study we demonstrated that intraperitoneal injection of creatine significantly decreased arrhythmias (ventricular premature beats and ventricular tachycardias) and early deaths after an AMI with respect to control animals or animals injected with an inert analogue of creatine. The present results show that creatine, while having no effects on cardiac electrical and mechanical activities, significantly increased the ventricular fibrillation threshold after an AMI in isolated rat hearts. A decreased activation of the TRPC3/C7 channels during the early phase of AMI can underlie the antiarrhythmic action of creatine. This can be explained as follows: We

have shown that ATP, released during the early phase of an AMI, activates, via a complex signaling cascade, the cationic (nonspecific) channel TRPC3/C7.[6] In the presence of excess creatine, ecto-nucleoside diphosphate kinases, NDPK, could significantly decrease the (released) interstitial ATP concentration by transferring phosphate groups between ATP and creatine to form creatine phosphate and ADP which is inactive on the TRPC3/C7 channel. Therefore, the depolarizing arrhythmogenic current through TRPC3/C7 channel will be significantly diminished and as a consequence, fewer arrhythmias occur.

To our knowledge, antiarrhyhthmic actions of creatine have been only reported by our laboratories. However, several studies suggest a cardioprotective action for creatine phosphate (CrP) since, through NDPK it can serve as a buffer for ATP in energy-depleted hearts.[13-15] Another study proposed that CrP could have an antiarrhythmic action due to its capacity to reduce degradation of membrane phospholipids thus diminishing lisophosphoglyceride production (LPG).[16] It has been shown that LPG accumulates in the ischemic zone causing electrical instability and arrhythmias.[17]

CONCLUSIONS

We may conclude that creatine, by increasing the ventricular fibrillation threshold during an acute myocardial infarction, could have an antiarrhythmic action. Although more preclinical studies are still necessary, our results suggest that creatine could be used as a therapeutic agent to prevent arrhythmias during ischemia or reperfusion.

REFERENCES

1. Vassort G. Adenosine 5'-triphosphate: a P2-purinergic agonist in the myocardium. Physiol. Rev. 2001; 81:767-806.
2. Kuzmin AL, Lakomkin VL, Kapelko VI, Vassort G. Interstitial ATP level and degradation in control and postmyocardial infarcted rats. Am. J. Physiol. 1998; 275:C766-71.
3. Kuzmin AL, Lakomkin VL, Vassort G. Effects of preconditioning on myocardial interstitial levels of ATP and its catabolites during regional ischemia and reperfusion in the rat.: Basic Res. Cardiol. 2000; 95:127-136.
4. Coade SB, Pearson JD. Metabolism of adenine nucleotides in human blood. Circ. Res. 1989; 65:531-7.
5. Vassort G, Puceat M, Alvarez J. Regulation of cardiac activity by ATP, a purine agonist. In: Molecular Physiology and Pharmacology of Cardiac Ion Channels. Edited by M. Morad et al; Kluwer Acad. Publ. pp: 231-8, 1996.
6. Alvarez JL, Coulombe A; Cazorla O et al. ATP/UTP activate cation-permeable channels with TRPC3/7 properties in rat cardiomyocytes. Amer. J. Physiol. Heart Circ. Physiol. 2008; 295:H21-8.
7. Vassort G, Alvarez JL. TRP, transient receptor potential, a large family of new channels of which several are involved in cardiac arrhythmia. Can. J. Physiol. Pharmacol. 2009; 87: 100-7.
8. Vassort G, Alvarez Jl. TRP channels in cardiac arrhythmia: their role during purinergic activation induced by ischemia. In: Heart Rate and Rhythm: Chapter 31. Molecular Basis, Pharmacological Modulation and Clinical Implications. Edited by: O. Tripathi, U. Ravens, M. Sanguinetti. Springer Verlag. pp: 563-579, 2011.
9. Vassort G, Bideaux P, Alvarez J. Could early ischemic arrhythmia triggered by purinergic activation of the transient receptor potential channels be prevented by creatine? Exp. Clin. Cardiol. 2010; 15:e104-8.
10. Bessman SP, Geiger PJ. Transport of energy in muscle: the phosphorylcreatine shuttle. Science 1891; 221: 448-52.
11. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. Physiol. Rev. 2000; 80: 1107-1213.
12. Cooper R, Naclerio F, Allgrove J, Jiménez A. Creatine supplementation with specific view to exercise/sports performance: an update. J. Int. Soc. Sports Nutr. 2012; 9: 33 [http://www.jissn.com/content/9/1/33].
13. Fagbemi O, Kane KA, Parratt JR. Creatine phosphate suppresses ventricular arrhythmias resulting from coronary artery ligation. J. Cardiovasc. Pharmacol. 1998; 4:53-8.
14. Hearse DJ, Tanaka K, Crome R, Manning AS. Creatine phosphate and protection against reperfusion-induced arrhythmias in the rat heart. Eur. J. Pharmacol. 1986; 131:21-30.
15. Sharov VG, Afonskaya NI, Ruda MY, et al. Protection of ischemic myocardium by exogenous phosphocreatine (neoton): pharmacokinetics of phosphocreatine, reduction of infarct size, stabilization of sarcolemma of ischemic cardiomyocytes, and antithrombotic action. 1986; Biochem. Med. Metab. Biol. 35:101-14.
16. Anyukhovsky EP, Javadov SA, Preobrazhensky AN, Beloshapko GG, Rosenshtraukh LV, Saks VA. Effect of phosphocreatine and related compounds on the phospholipid metabolism of ischemic heart. 1986; Biochem. Med. Metab. Biol. 35:327-34.
17. Corr PB, Saffitz JE, Sobel BE. Lysophospholipids, long chain acylcarnitines and membrane dysfunction in the ischaemic heart. Basic Res. Cardiol. 1987; 82 (Suppl 1):199-208.

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