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Molecular, Biological, Physiological and Clinical Aspects of Melatonin

Melatonin, given at the time of reperfusion, prevents ventricular arrhythmias in isolated hearts from fructose-fed rats and spontaneously hypertensive rats

Abstract: Melatonin reduces reperfusion arrhythmias when administered before coronary occlusion, but in the clinical context of acute coronary syndromes, most of the therapies are administered at the time of reperfusion. Patients frequently have physiological modifications that can reduce the response to therapeutic interventions. This work determined whether acute melatonin administration starting at the moment of reperfusion protects against ventricular arrhythmias in Langendorff-perfused hearts isolated from fructose-fed rats (FFR), a dietary model of metabolic syndrome, and from spontaneous hypertensive rats (SHR). In both experimental models, we confirmed metabolic alterations, a reduction in myocardial total antioxidant capacity and an increase in arterial pressure and NADPH oxidase activity, and in FFR, we also found a decrease in eNOS activity. Melatonin (50 μM) initiated at reperfusion after 15-min regional ischemia reduced the incidence of ventricular fibrillation from 83% to 33% for the WKY strain, from 92% to 25% in FFR, and from 100% to 33% in SHR ($P = 0.0361$, $P = 0.0028$, $P = 0.0013$, respectively, by Fisher's exact test, n = 12 each). Although, ventricular tachycardia incidence was high at the beginning of reperfusion, the severity of the arrhythmias progressively declined in melatonin-treated hearts. Melatonin induced a shortening of the action potential duration at the beginning of reperfusion and in the SHR group also a faster recovery of action potential amplitude. We conclude that melatonin protects against ventricular fibrillation when administered at reperfusion, and these effects are maintained in hearts from rats exposed to major cardiovascular risk factors. These results further support the ongoing translation to clinical trials of this agent.

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Key words: arrhythmias, melatonin, metabolic syndrome, reperfusion injury, spontaneously hypertensive rats

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Introduction

Different administration schemes as well as several animal models have documented the cardioprotective actions of melatonin, mainly against ischemia–reperfusion injury [1]. Most of the studies that confirmed the protective effect of acute melatonin administration were performed in relatively healthy hearts, and only three of them included groups in which the drug was administered at the time of reperfusion [2–5]. We have recently described that melatonin, perfused prior to coronary occlusion and throughout the entire period of ischemia and reperfusion, inhibited transmembrane potential modification during ischemia and reduced reperfusion arrhythmias [6]. However, in the clinical scenario, the interventions would be initiated after ischemia onset, and the drugs would reach the tissue at risk only at the time of reflow [7]. The seminal work of Tan et al. [2] already showed that melatonin reduces irreversible ventricular fibrillation when administered 2 min prior to reperfusion, but electrophysiological changes associated with this effect remain unknown.

The use of cardiovascular risk factor models has been recently emphasized in the recommendations to improve progress in protection against ischemia–reperfusion injury and facilitate translation of promising therapies from preclinical to clinical use [8, 9]. Chronic administration of melatonin in animal models of cardiovascular risk factors prevented some deleterious effects, mainly those related to the increase oxidative stress [1], but the protection against ischemia/reperfusion injury in the acute administration scenario in hearts modified by the chronic exposure to the risk factors has not been tested [10–12]. The latter is of particular interest for melatonin because there are two phase II clinical trials (NCT01172171 and NCT00640094) ongoing to assess melatonin effectiveness as adjuvant in the treatment of acute myocardial infarction [13, 14]. Despite the safe profile of this drug, some data are missing in the translational pathway like what happens in other animal models or in the presence of a pre-existing risk factor at the moment of the intervention.

Herein, we investigated whether the electrophysiological modifications induced by cardiovascular risk factors

observed in two different rat models were improved by acute administration of 50 μM melatonin given at the onset of reperfusion [15, 16]. We chose two experimental models because they better represent the cardiovascular risk factors commonly found in patients that suffer from acute coronary syndrome. Furthermore, metabolic syndrome and myocardial hypertrophy are risk factors for arrhythmic sudden cardiac death [17, 18]. Despite the fact that both conditions are very complex from a pathophysiological point of view, they share oxidative stress as a potential target to melatonin protection [12, 19]. We tested melatonin effects against ischemia-/reperfusion-induced arrhythmias, because despite their reversibility, they can be a manifestation of lethal ischemia/reperfusion injury and they are also a short- and long-term prognostic indicator of mortality [20]. In addition, we studied the transmembrane potential to evaluate local electrophysiological modification during the intervention.

Methods

Animal models

All procedures were approved by the local Institutional Animal Care and Use Committee, which are in agreement with the Guide for Care and Use of Laboratory Animals (National Academy Press, 1996). Age-matched male rats were housed in metal cages under conditions of controlled temperature and humidity, with food and water ad libitum, and exposed to a cycle of 12 hr of light and 12 hr of darkness. The experiments were performed between 12:00 and 15:00 in three-month-old Wistar Kyoto (WKY) rats, and spontaneously hypertensive rats (SHR) and WKY rats with metabolic syndrome induced chronic administration of fructose in the drinking water (10% w/v for the last 6 wk before sacrifice, FFR), as previously described [15].

Systolic blood pressure measurement

The systolic blood pressure (SBP) was monitored indirectly in conscious prewarmed slightly restrained rats by the tail-cuff method and recorded using a Koda2 device (Kent Scientific Corporation, USA) the day before the isolated heart experiments. The rats were trained on the apparatus several times before measurement.

Biochemical determinations

Blood was collected from the abdominal aorta into heparinized tubes at the moment of hearts extraction after 19 ± 1.5 hr of overnight fasting. Plasma obtained after centrifugation was frozen at 70°C until assayed. Plasma insulin was assayed by ELISA DSL-10-1600 ActiveTM, Diagnostic System Laboratories, INC (Webster, TX, USA) in a multiwell plate reader Rayto and expressed as μU/mL. Plasma glucose, triglycerides, and HDL cholesterol levels were assayed using a commercial colorimetric method (Wiener Lab., Rosario, Argentina) and expressed as mg/dL. We calculated the homeostasis model assessment of insulin resistance (HOMA-IR) adapted to rat s using the formula [21]:

$$\frac{(\text{fastin plasma insulin} \times \text{fasting plasma insulin})}{2.43}$$

Markers of oxidative stress

Arterial NADPH oxidase activity was measured according to previously described methods by the lucigenin-derived chemiluminescence assay in segments dissected from the abdominal aorta of the animals immediately after slaughter and incubated in Jude–Krebs buffer [5, 7, 18]. β-NADPH was then added (as substrate) and chemiluminescence was measured continuously for 3 min on a microplate fluorometer (Fluoroskan Ascent FL, Thermo LabSystems, Waltham, MA, USA). Enzymatic activity was adjusted to the weights of the arteries and was expressed in counts per minute per milligram of tissue (cpm/mg).

The activity of the Ca^{2+}-/calmodulin-dependent endothelial nitric oxide synthase (eNOS) enzyme was measured in homogenates of mesenteric arteries by conversion of L-[3H] arginine in L-[3H] citrulline, as previously described [22]. Calcium dependent NOS activity was calculated as the difference between activities in the presence or absence of Ca^{2+}/calmodulin. Values were corrected according to protein content (Bradford method) in the homogenates and incubation time and expressed as dpm/mg protein/min. The material obtained from each animal was processed independently.

In five additional hearts from the corresponding experimental models, we measured the total antioxidant capacity (TAC) in myocardial samples taken from the region corresponding to the territory served by the anterior descending coronary artery. The samples were weighed (100 mg), dried with filter paper, transferred to Eppendorf tubes containing phosphate buffered saline, pH 7.4, and stored at −75°C until processing. The technique was previously described [6, 23]. In brief, the preformed radical $ABTS^{\bullet+}$, monocation of 2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) generated by oxidation of ABTS with potassium persulfate, was reduced with hydrogen-donating antioxidants. Ventricular homogenates (100 mg/mL) were compared using ascorbic acid (1 mM) as reference of total antioxidant capacity. All samples were read at 600 nm with a UV visible Spectrometer model Helios Gamma, Helios Delta (Unicam instruments, Cambridge, UK), after 18 min of incubation at 37°C. The results are expressed in ascorbate equivalent per liter (Ae/L).

Langendorff-perfused rat hearts

Rats were sacrificed by cervical dislocation under anesthesia with 60 mg/kg ketamine and 0.1 mg/kg acepromazine by intraperitoneal injection. The hearts were rapidly excised and kept at 4°C until being connected to perfusion system, always less than 3 min. The hearts were perfused at constant pressure of 80 cm H_2O for WKY and FFR or 110 cm H_2O in the case of SHR, according to a pilot study performed in two rats of each strain to determine the perfusion pressure required to match the coronary flow per gram of tissue.

All hearts were initially perfused with a modified Krebs–Henseleit solution containing (in mM): 121 NaCl, 25 $NaHCO_3$, 1.2 Na_2HPO_4, 5 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 11 glucose. When equilibrated with 5% CO_2 in O_2 at 36.5 ± 0.5°C, the pH was 7.4 ± 0.02. To obtain one liter of 50 μM solution, melatonin was dissolved in 1.5 mL ethanol and then added to Krebs–Henseleit solution. We choose this melatonin dose based on previous studies [5, 6]. Care was taken not to expose the solutions to light. Melatonin was purchased from Sigma-Aldrich (Saint Louis, MO, USA).

The coronary flow was measured throughout the experiment. It was used as an index of adequate perfusion and as a criterion to assess the efficiency of coronary ligation. A reduction of at least 25% during occlusion was considered satisfactory. To validate reproducibility, we re-occluded the artery at minute 10 of reperfusion and perfused Evans blue, and after 1 hr of cooling at −20°C, we sliced the ventricles transversely from apex to base in to 2-mm slices. The colored zones other than blue were considered the area at risk. Slices were photographed for planimetric analysis (ImageJ 1.43, Wayne Rasband, National Institute of Health, Bethesda, MD, USA) and weighed for adjusted expression of the area at risk. We only included hearts with an ischemic area greater than 40% of the ventricles to guarantee reproducibility of reperfusion arrhythmias incidence [24].

Reperfusion arrhythmias and action potentials

After 20 min of stabilization, we continuously obtained surface electrograms equivalent of lead II and epicardial transmembrane potential using a Hewlett-Packard 1500A and a custom-made microelectrode amplifier, respectively. Both signals were digitized with an analog to digital converter NI PCI-6221 (National Instruments, Austin, TX, USA) and recorded using LabView Signal Express 2.5. After a period of 10 min pre-ischemia, hearts underwent 15 min of regional ischemia by ligation of the left anterior descending coronary artery. We evaluated the effect of melatonin administration during reperfusion in respect to the vehicle. Two groups for each strain were determined as follows: (i) WKY, (ii) WKY-M, (iii) SHR, (iv) SHR-M, (v) FFR and (vi) FFR-M (n = 12 per group). Ventricular arrhythmias were classified according to the Lambeth convention [25]. We evaluated the incidence and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF). We also evaluated the arrhythmias severity each minute using the following score: 0 – sinus rhythm, 1 – premature ventricular beats or bigeminy, 2 – Salvos, 3 – nonsustained VT (<30 s) 4 – sustained VT (>30 s) or VF [26]. We analyzed the following parameters of epicardial transmembrane potentials: action potential amplitude, resting potential, action potential duration at 50% and 90% of repolarization, and the maximum rate of depolarization ($\delta V/\delta t$ max).

Myocardial hypertrophy

The degree of myocardial hypertrophy was evaluated based on the relative heart weight in respect to body weight (RHW, mg/g).

Statistical analysis

Data were expressed as mean ± S.E.M., and statistical analysis was performed using ANOVA or two-way repeated-measures ANOVA followed by Bonferroni post-test and Fisher's exact test, as appropriate. Variables not normally distributed were analyzed using Kruskal–Wallis test followed by Dunn post-test.

Results

We confirmed the presence of metabolic syndrome in both experimental models (see Tables 1 and 2). In FFR, we found an increase in body weight, fasting glucose, HOMA index, and dyslipidemia, and a slight increase in systolic blood pressure. SHR showed different diagnostic criteria for metabolic syndrome. The body weight was lower than the others groups, but they showed higher arterial pressure values, higher triglycerides, lower HDL, and also an increase in the HOMA index. Both models showed myocardial hypertrophy, which was more pronounced in the SHR hearts (Table 2).

In both models, we found an increase in oxidative stress markers (Table 1). The NADPH oxidase activity was increased in FFR around four times and in SHR around ten times. Interestingly, only FFR showed reduced eNOS activity. The myocardial total antioxidant capacity was reduced in a similar level in both experimental models (Table 1).

Coronary flow was similar for all groups, and the degree of reduction was significant and stable after coronary

Table 1. Metabolic parameters of the experimental models of cardiovascular risk factors

	WKY	FFR	SHR
Plasma glycemia (mg/dL)	87.8 ± 2.6	112.5 ± 2.8*	92.6 ± 2.2
Plasma insulinemia (μU/mL)	0.1082 ± 0.0002	0.1246 ± 0.0003*	0.2296 ± 0.0004*^
HOMA-IR	3.9 ± 0.06	5.77 ± 0.10*	8.78 ± 0.14*^
Plasma triglycerides (mg/dL)	67.3 ± 1.7	80.3 ± 2.5*	123.4 ± 2.0*^
Plasma HDL (mg/dL)	22.5 ± 0.5	19.3 ± 0.6*	12.2 ± 0.5*^
NADPH oxidase (cpm/mg)	15.5 ± 2.1	66.1 ± 3.2*	154.3 ± 6.5 *^
eNOS (dpm. mg prot/min)	82.3 ± 1.3	60.0 ± 1.3*	80.7 ± 2.6 ^
TAC (Ae/L)	0.418 ± 0.011	0.254 ± 0.025*	0.271 ± 0.019*

Values correspond to mean ± S.E.M. Pooled data from n = 24 for each column, except in TAC where n = 5 for each column. *$P < 0.001$ versus WKY. ^$P < 0.001$ versus FFR.

Table 2. Biometric data of the experimental models of cardiovascular risk factors

	WKY	FFR	SHR
Body weight (g)	321 ± 4.5	345 ± 5.2*	287 ± 3.9*ˆ
Heart weight (mg)	1219.8 ± 14.5	1552.5 ± 16.2 *	1607.2 ± 11.7*
Relative heart weight (mg/g)	3.8 ± 0.1	4.5 ± 0.2*	5.6 ± 0.1 *ˆ
Mean Arterial pressure (mmHg)	81.6 ± 1.2	84.1 ± 2.0	122.4 ± 1.1*ˆ
Systolic blood pressure (mmHg)	118 ± 0.8	136 ± 2.9*	182.1 ± 1.0*ˆ

Values correspond to mean ± S.E.M. Pooled data from n = 24 for each column. *$P < 0.001$ versus WKY. ˆ $P < 0.001$ versus FFR.

occlusion. Melatonin did not modify the recovery of coronary flow during reperfusion (Table 3). There was no difference in basal heart rate between the groups, and all suffered a reduction around 20–40 beats/min during ischemia (Table 3). During reperfusion, most of the heart treated with the vehicle developed ventricular arrhythmias, which interfered with comparative heart rate analysis; however, melatonin-treated hearts recovered the pre-ischemic frequency (Table 3). The ischemic area measured at the end of the protocol did not differed between the groups, and the corresponding values were as follows: WKY 45.2 ± 1.4; WKY-M 44.9 ± 1.2; FFR 45.1 ± 1.2; FFR-M 44.4 ± 1.6; SHR 43.9 ± 1.3; and SHR-M 44.6 ± 1.4.

Ventricular arrhythmias occurred at the onset of reperfusion in the vehicle-treated hearts (Fig. 1). The administration of melatonin reduced the incidence and duration of VF (Fig. 2 and Table 4). Melatonin did not modify the incidence or duration of VT between groups (Table 4). However, the lack of effect in VT duration did not clearly reflect that in melatonin-treated groups, and this was due to the predominance of sinus rhythm during reperfusion, whereas in the control group, this was due to the predominance of sustained arrhythmias. These results were supported by the reduction in the severity score in the melatonin-treated hearts (Fig. 3), which also indicates that the anti-arrhythmic effect was sustained, because vehicle-treated hearts maintained a high level of severity.

Analysis of the transmembrane potential of epicardial cardiomyocytes identified that the three strains of rats had similar behavior to the ischemia–reperfusion injury. The amplitude was very similar in all groups during the protocol, except for a marked recovery present in SHR-M group at the beginning of reperfusion (Fig. 4A). The resting potential and $\delta V/\delta t_{max}$ did not differ between groups (Fig. 4B,C). As for the action potential duration at 50% and 90% of repolarization, SHR strain exhibited a prolongation during pre-ischemia with respect to the WKY and FFR groups ($P < 0.01$). However, during ischemia, this prolongation was only seen for APD_{50} values ($P < 0.05$). Melatonin reduced the action potential duration especially at the beginning of reperfusion, but this effect was less marked in the SHR strain (Fig. 4D,E).

Discussion

The main findings in this study are that acute melatonin administration during reperfusion exerts a strong protective effect against ventricular arrhythmias and that this protection persists in hearts from animals exposed to cardiovascular risk factors included in the definition of metabolic syndrome, which have an increase in oxidative stress markers previous to ischemia–reperfusion.

To our knowledge, this is the first study that confirms acute melatonin protective effects against ischemia/reperfusion in hearts isolated from rats exposed to cardiovascular risk factors. This pharmacological time frame was previously explored in healthy rats' hearts by Tan et al. [2] in respect to reperfusion arrhythmias using 10 μM melatonin and by Lochner et al. [4] and Genade et al. [5] in respect to infarction, both using 50 μM melatonin. Our results in isolated hearts from WKY are in agreement with these studies demonstrating that melatonin reduces ischemia/reperfusion injury in normal healthy rat hearts. However, there is an increasing need for effective cardioprotective strategies that can reduce reperfusion injury in hearts previously exposed to cardiovascular risk factors, which may interfere with cardioprotection [27, 28]. There is good

Table 3. Coronary flow and heart rate during the experimental protocol

	Coronary flow (mL/g)			Heart rate (beats/minutes)		
	Pre-ischemia	Prereperfusion	10 min of reperfusion	Pre-ischemia	Prereperfusion	10 min of reperfusion
WKY	6.9 ± 0.4	4.1 ± 0.4 *	5.4 ± 0.5	302 ± 15	262 ± 16	282 ± 19 (3)
WKY-M	6.8 ± 0.5	4.0 ± 0.3 *	5.5 ± 0.4	296 ± 16	260 ± 15	269 ± 13 (11)
FFR	7.1 ± 0.3	4.3 ± 0.5 *	5.5 ± 0.4	289 ± 20	261 ± 19	281 ± 20 (3)
FFR-M	6.9 ± 0.4	4.1 ± 0.2 *	5.3 ± 0.3	304 ± 16	266 ± 18	290 ± 12 (12)
SHR	6.8 ± 0.4	4.2 ± 0.5 *	5.4 ± 0.2	297 ± 15	259 ± 14	279 ± 19 (3)
SHR-M	6.9 ± 0.5	4.2 ± 0.4 *	5.6 ± 0.3	291 ± 13	269 ± 10	284 ± 14 (10)

All values represent mean ± S.E.M. of n = 12. The heart rate during reperfusion corresponds to the number of hearts in sinus rhythm indicated in the parenthesis. *$P < 0.01$ versus pre-ischemia.

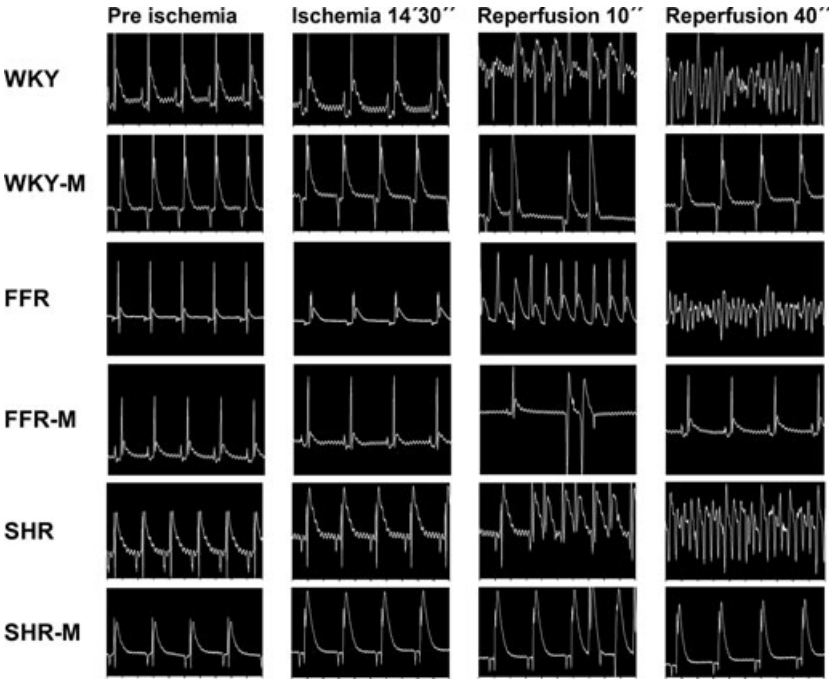

Fig. 1. Surface electrograms obtained at comparable times from each group in the experimental periods indicated on top. All the pictures correspond to 1 s of the recording.

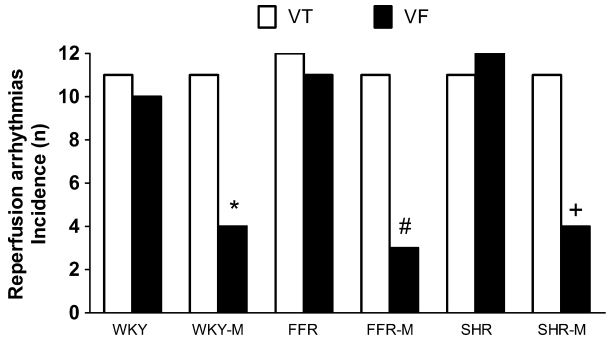

Fig. 2. Incidence of ventricular arrhythmias during reperfusion. White bars indicate the number of hearts that developed ventricular tachycardia (VT), and black bars indicate those that suffered ventricular fibrillation (VF) for each group. $*P = 0.0361$ WKY versus WKY-M; $^{\#}P = 0.0028$ FFR versus FFR-M; $^{+}P = 0.0013$ SHR versus SHR-M by Fisher's exact test, n = 12 each.

Table 4. Reperfusion arrhythmias duration

	Ventricular tachycardia	Ventricular fibrillation
WKY	33.0 (0–41)	459.0 (228–555)
WKY-M	31.5 (28–55)	0.0 (0–28)*
FFR	40.5 (12–75)	466.0 (198–548)
FFR-M	49.5 (19–77)	0.0 (0–61)#
SHR	55.0 (20–85)	415.5 (211–477)
SHR-M	39.5 (16–97)	0.0 (0–56)+

All values correspond to the median (1st quartile–3rd quartile) expressed in seconds. $*P < 0.01$ WKY versus WKY-M; $^{\#}P < 0.01$ FFR versus FFR-M; and $^{+}P < 0.01$ FFR versus FFR-M analyzed by Kruskal–Wallis test followed by Dunn post-test.

evidence that chronic melatonin therapy reduces reperfusion injury in hearts of animal exposed to cardiovascular risk factors like cardiomyopathic hamsters, chronically hypoxic rats, and diet-induced obesity [10–12]. Of translational relevance, we demonstrate that melatonin is effective when given upon reperfusion even under pathological conditions frequently associated with the clinic scenario of ischemia/reperfusion injury.

Most of the studies indicate an increase in the deleterious effect of ischemia/reperfusion in experimental models of cardiovascular risk factors, but others did not or even have found a decrease [27, 29, 30]. We have previously demonstrated that these animal models present higher vascular and myocardial oxidative stress status [22, 31]. Here, we did not find any difference in reperfusion arrhythmias between the experimental models (Figs 2 and 3). We attribute this to the high incidence and severity found in all groups treated with the vehicle. Although this was expected due to the area submitted to ischemia and reperfusion, it could be seen as a possible limitation of this study. However, here, we show that these physiological modification in oxidative stress (see Table 1) added to the one generated due to myocardial ischemia/reperfusion did not interfered with melatonin acute protection.

An explanation for the acute melatonin protective effects against ischemia–reperfusion injury could be its direct free radical scavenging actions [32, 33], which could rapidly protect against the abrupt generation of free radical at reperfusion. Furthermore, several metabolites formed, when melatonin neutralizes damaging reactants, are themselves scavengers, suggesting an enhanced efficacy when used in pathological conditions associated with increased oxidative stress [34–38]. Our results are consistent with this point of view and add new information with respect to the previous studies in which chronic melatonin administration attenuated oxidative stress in diseased

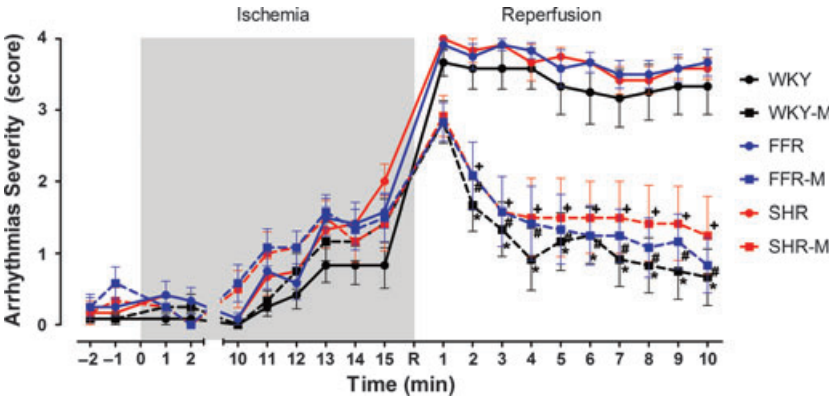

Fig. 3. Arrhythmias severity score. Circles with continuous connecting lines indicate vehicle-treated hearts, and squares with dash connecting lines indicate melatonin-treated hearts. Black, blue, and red are used to indicate WKY, FFR, and SHR, respectively. $*P = 0.05$ WKY versus WKY-M; $^{\#}P = 0.05$ FFR versus FFR-M; $^{+}P = 0.05$ SHR versus SHR-M by two-way repeated-measures ANOVA.

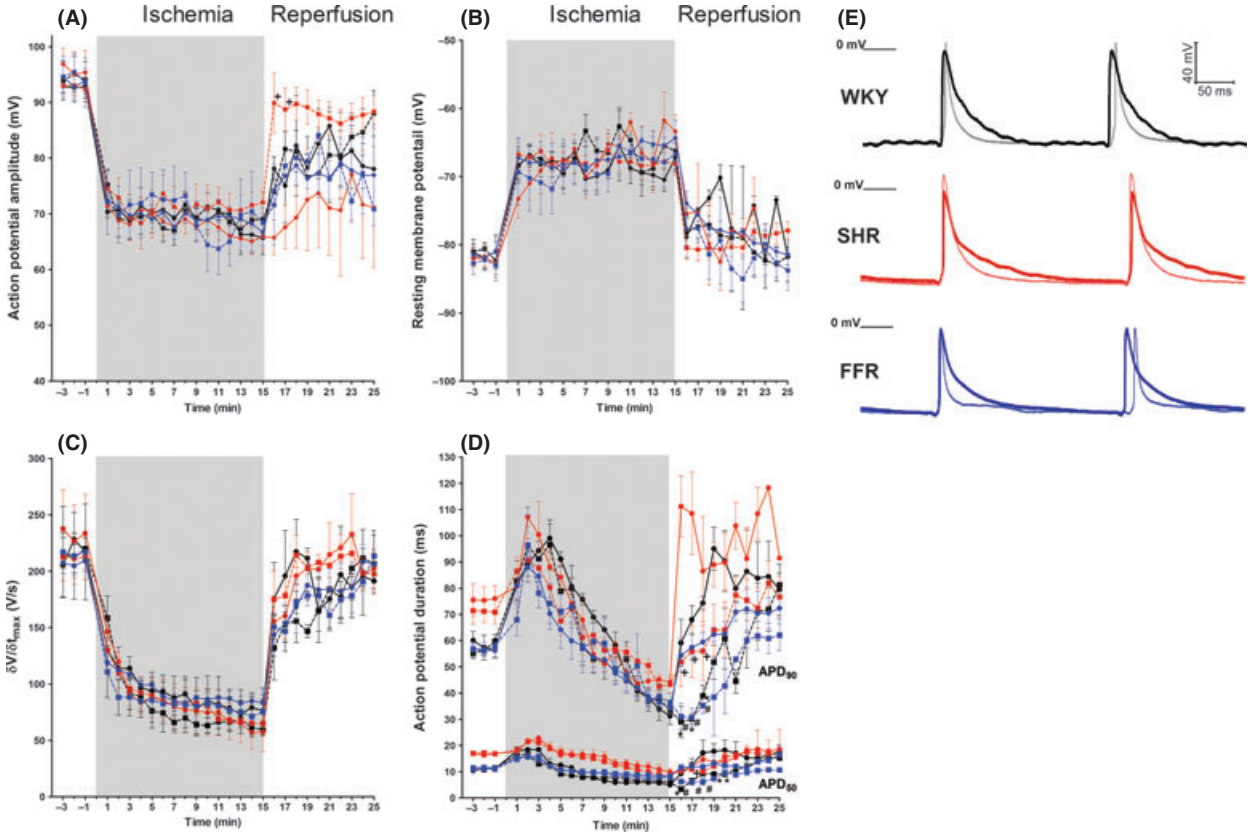

Fig. 4. Transmembrane potential variables during ischemia/reperfusion protocol for each group. (A) Action potential amplitude. (B) Resting membrane potential. (C) Maximum rate of depolarization ($\delta V/\delta t_{max}$). (D) Action potential duration at 50% and 90% of repolarization (APD_{50} and APD_{90}, respectively). Circles with continuous connecting lines indicate vehicle-treated hearts, and squares with dash connecting lines indicate melatonin-treated hearts. Black, blue, and red are used to indicate WKY, FFR, and SHR, respectively. $*P = 0.05$ WKY versus WKY-M; $^{\#}P = 0.05$ FFR versus FFR-M; $^{+}P = 0.05$ SHR versus SHR-M by two-way repeated-measures ANOVA. (E) Representative traces obtained during the second minute of reperfusion from a heart in sinus rhythm. The thicker and darker traces correspond to vehicle-treated hearts.

models by increasing antioxidant defenses and reducing pro-oxidative systems. Although we did not test the inhibition of the opening of mitochondrial transition pore by melatonin, this is an unsuitable explanation for the anti-arrhythmic effect here described, because other investigations confirmed that acute treatment with cyclosporine A, an inhibitor of the mitochondrial transition pore opening, lacked protective effects against reperfusion arrhythmias in three animal models [39–41].

Melatonin protection against reperfusion arrhythmias was not affected by the time of administration, but its effects on epicardial action potentials presented a differential response relative to a previous report [6]. Recently, we associated the protective effect of melatonin against reperfusion arrhythmias when administered before

regional ischemia with the inhibition of action potential shortening at the end of this period, followed by a rapid recovery to pre-ischemic values during reperfusion [6]. In the present study, we show that the anti-arrhythmic effects were maintained when administered at the onset of reperfusion and were associated with a delay in the recovery from the action potential shortening, which was already established at the end of the ischemic period. This action potential shortening could attenuate calcium overload at the beginning of reperfusion and contribute to the reduction in ventricular fibrillation and other forms of sustained arrhythmias. The proposed anti-adrenergic action of melatonin could underlie this response [5]. The latter is in agreement with an action potential shortening and an anti-arrhythmic effect induced by β_1 blocker administration during reperfusion (unpublished result). This could be an indirect indication that both scavenger properties as well as receptor-mediated anti-adrenergic response could contribute to the cardioprotective effect of melatonin.

The specific details of the mechanisms involved in melatonin's protection during reperfusion remain to be clarified, but due to its safety profile and almost uniform protective effect, this compound has indicated that this is a promising therapeutic intervention against ischemia/reperfusion injury. Our experimental data on its efficacy under pathological conditions further contribute to the rationale of the clinical trials in progress [13, 14]. The two ongoing phase II clinical trials designed to assess melatonin effectiveness as adjuvant in the treatment of acute myocardial infarction are supported by the results described here.

In summary, the data obtained in this study confirm that melatonin maintained its anti-arrhythmic effect against reperfusion injury in isolated rat hearts resulting from major cardiovascular risk factors. Acute melatonin was effective against lethal reperfusion arrhythmias, especially VF. These effects were achieved when administered during reperfusion, which resembles the clinical situation where the intervention can only be performed after initiation of ischemia, and more precisely, as an adjuvant therapy to reperfusion. Notably, the protective effect was maintained in animals with higher levels of oxidative stress.

Authors' contribution

Emiliano Raúl Diez MD involved in the conception and design, acquisition of data, data analysis/interpretation, drafting of the manuscript, critical revision, and final approval of manuscript. Nicolás F. Renna MD, Natalia Jorgelina Prado, and Carina Lembo involved in the design, experimental studies, data analysis/interpretation, critical revision, and final approval of manuscript. Amira Zulma Ponce Zumino MD involved in the conception and design, data interpretation, critical revision, and final approval of manuscript. Marcela A. Vazquez PhD involved in the experimental studies, data analysis/interpretation, review, and final approval of manuscript. Roberto M. Miatello PhD involved in the conception and design, data interpretation, manuscript editing, review, and final approval of manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Infarct size expressed as % of the area at risk. Melatonin (50 μM) administration during the initial 30 min of reperfusion reduced infarct size produced by 30 min of regional ischemia and 120 minutes of reperfusion from 30.0 ± 2.2 to 7.1 ± 1.3 in Wistar Kyoto rats (WKY), from 31.6 ± 4.0 to 9.8 ± 2.6 in FFR, and from 32.7 ± 2.9 to 12.8 ± 2.0 in SHR (all values are mean percentage of the area at risk $\pm$ SEM, $n = 6$; *** $P < 0.001$ melatonin-treated versus vehicle-treated hearts).