*J. Pineal Res. 2008*
**Doi:10.1111/j.1600-079X.2008.00643.x**


**Journal of Pineal Research**

# A novel electrophysiologic effect of melatonin on ischemia/reperfusion-induced arrhythmias in isolated rat hearts

Abstract: Reperfusion after a short period of cardiac ischemia triggers ventricular arrhythmias attributable to ionic imbalance and oxidative stress. Melatonin offers some degree of protection, but its effects on the cardiac action potentials are unknown. We evaluated the effects of 5, 10, 20 and 50 $\mu$M melatonin in isolated perfused rat hearts subjected to 10 min of regional ischemia. ECG and membrane potentials were synchronously displayed. After 15 min of reperfusion, total antioxidant capacity (TAC) was determined. Melatonin did not change the ischemic depolarization nor the action potential amplitude depression, but at the end of ischemia the action potential duration (APD) decreased in control and 5 $\mu$M melatonin-treated hearts. By contrast, it returned to preischemic levels in hearts given 20 and 50 $\mu$M melatonin. Melatonin reduced the incidence of reperfusion arrhythmias from 100% in control to 50% in 5 and 10 $\mu$M, to 40% in 20 $\mu$M and 30% in 50 $\mu$M hearts. TAC values were higher at all melatonin concentrations. We conclude that melatonin reduced the incidence of reperfusion arrhythmias because of its antioxidant effects. In addition, at 20 and 50 $\mu$M lengthened APD and promoted an improved protection. This latter effect should be considered when in vivo applications of melatonin are considered.

**Emiliano Raúl Diez[1,2], Laura Vanina Prados[1], Adriana Carrión[1], Zumino Amira Zulma Ponce[1] and Roberto Miguel Miatello[2]**

[1]Department of Physiology, Faculty of Medical Sciences, National University of Cuyo; [2]Institute for Medical and Biological Experimental Research of Cuyo (IMBECU)–National Council of Scientific and Technological Investigation (CONICET), Mendoza, Argentina

Key words: antioxidant, arrhythmias, electrophysiology, ischemia-reperfusion injury, melatonin, rat, transmembrane action potential

Address reprint requests to Emiliano Diez, Department of Physiology, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Av. Libertador 80, Centro Universitario 5500, Mendoza, Argentina.
E-mail: diez.emiliano@fcm.uncu.edu.ar

Received June 17, 2008;
accepted September 30, 2008.

## Introduction

It is known that reperfusion following a short period of ischemia generates oxidative stress as a result of an abrupt burst of free radicals (FR) and other reactive substances. Both oxidative stress and the associated intracellular calcium overload during early reperfusion are believed to trigger severe ventricular arrhythmias [1, 2]. Although several factors may be involved, this pathological process is not yet fully understood. Several studies have shown that the incidence and the severity of reperfusion arrhythmias **(**RA**)** can be modified by procedures that reduce the oxidative stress and/or modulate the calcium overload, the acid production or the action potential duration (APD) [3–8].

Melatonin (N-acetyl-5-methoxytryptamine) is a pineal secretory product that participates in numerous physiological and neuroendocrine functions with remarkable antioxidant effects as scavenger of FR including peroxynitrite, hydroxyl and peroxyl radicals [9–13]. Besides these properties, it also inhibits peripheral sympathetic responses, decreases the availability of voltage-operated $Ca^{2+}$ channels and stimulates the $Ca^{2+}$-dependent ATPase in cardiac sarcolemma [14, 15]. Recent studies report that melatonin infused during an ischemia-reperfusion period also prevents the occurrence of RA [16–21]. Furthermore, because of its cardioprotective effects, a phase-2 clinical trial has been designed using melatonin as an adjunct in angioplasty therapy of acute myocardial infarction [22]. These evidences have been reviewed recently by Tengattini et al. [23]. However, few reports have described changes induced by melatonin on cardiac electrophysiological variables.

In the experiments here reported, we studied the effects of melatonin on the surface electrogram and the ventricular membrane potential of isolated rat hearts subjected to an ischemia-reperfusion protocol. We also determined melatonin′s antioxidant effect in myocardial tissue and its influences on the heart rate (HR) and the coronary flow (CF).

## Materials and methods

### Animals and experimental procedures

Female Sprague–Dawley rats weighing 280–330 g were used according to availability and guidelines of local warehouse. They were housed in metal cages 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 14:00 and 17:00, previous confirmation of diestrus stage of estrous cycle in all the cases. The animals were killed by cervical dislocation according to the proto-

cols accepted by institutional guidelines (Committee on Ethics of Animal Experimentation, Faculty of Medical Sciences, National University of Cuyo). The hearts were rapidly removed and placed in oxygenated, 4°C Krebs–Henseleit buffer for removal of extracardiac tissue. In less than 4 min they were mounted on the aortic cannula of the Langendorff perfusion setup. The perfusion medium (modified Krebs–Henseleit solution) contained (mM) 121 NaCl, 5 KCl, 2 $CaCl_2$, 1.2 $MgSO_4$, 1.2 $NaHPO_4$, 25 $NaHCO_3$ and 5.5 glucose. When equilibrated with 5% $CO_2$ in $O_2$, the pH was 7.4 ± 0.05, at 36 ± 0.5°C. To facilitate microelectrode impalements, spontaneously beating hearts were placed horizontally into a double walled tissue chamber.

Five experimental series were performed: control (n = 11) and 5, 10, 20 or 50 $\mu$M melatonin (n = 10 each). Both control and melatonin media contained 0.5 mL/L ethanol. Melatonin was purchased from Sigma-Aldrich Cat. M5250 (Saint Louis, MO, USA) and perfused from the beginning of the experiment. The experimental protocol consisted of 20 min of equilibration followed by data recording for 15 min preischemia (PI). Regional ischemia was induced by tightening a ligature around the anterior descending coronary artery with a 6/0 silk thread for 10 min. For examination of the time course of the changes, we subdivided ischemia in: early (EI) from 0 to 3 min, and late (LI) from 6 to 10 min of ischemia. The CF was used as an index of proper perfusion, and for the evaluation of possible changes in coronary arterial tone at PI, ischemia and reperfusion. A reduction of total CF by 25% or more, after occlusion, was corroborated [17].

### Electrophysiological properties and criteria for arrhythmias

The membrane potential was recorded with flexibly mounted glass microelectrodes from subepicardial ventricular cells and synchronously displayed with the surface electrogram (ECG), obtained from two epicardial electrodes placed in the conventional standard II lead. Microelectrodes were filled with 3 M KCl and had resistances of 10–15 MΩ. The recording equipment consisted of a custom made microelectrode amplifier, a Tektronix 565 oscilloscope (Tektronix, Beaverton, OR, USA) and a C4 Grass camera (Grass Instruments, Quincy, MA, USA). Both signals were photographed from the screen of the oscilloscope. The following action potential characteristics were measured: resting potential (RP), action potential amplitude (APA) and action potential duration, determined from the onset of the fast rising upstroke to 90% of repolarization ($APD_{90}$). The HR as well as RA was analyzed from the ECG and the cell recordings. The type of arrhythmia was determined according to the Lambeth conventions [24]. In addition to the incidence of RA, also the severity was classified. When ventricular tachycardia and/or ventricular fibrillation lasted the entire reperfusion period it was classified as an irreversible reperfusion arrhythmia, in those hearts that developed ventricular tachycardia and/or ventricular fibrillation but recovered a sinus rhythm during reperfusion were referred as reversible reperfusion arrhythmia while hearts that maintained a sinus rhythm were referred to as no reperfusion arrhythmias. None of the hearts included in this study presented overt tachyarrhythmias prior to reperfusion.

### Evaluation of total antioxidant capacity in heart tissue

After 15 min of reperfusion some hearts were dried with filter paper and pieces from the left ventricle free wall (approximately 500 mg) were removed and transferred to Eppendorf tubes containing phosphate buffered saline, pH 7.4, at −75°C for processing. The total antioxidant capacity (TAC) was determined according to the technique described by Re et al. [25], modified by replacing trolox with ascorbic acid. The preformed radical ABTS•+, monocation of 2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. Ventricular homogenates (500 mg/mL) were compared using ascorbic acid (1 mmol/L) as reference of total antioxidant capacity. All samples were read at 600 nm with a UV-visible spectrometer model Helios gama, Helios delta (Unicam instruments, Cambridge, UK), after 18 min of incubation at 37°C. The results are expressed in ascorbate equivalent per litre (Ae/L). Because of the accuracy of the method, we randomly selected the samples for TAC determination and they were as follows: control n = 10; 5 $\mu$M melatonin n = 5; 10 $\mu$M melatonin n = 5; 20 $\mu$M melatonin n = 7; 50 $\mu$M melatonin n = 5.

### Statistical analysis

Data are expressed as means ± S.E.M. The results were analyzed with two-way ANOVA followed by the Student–Newman–Keuls post hoc test. The incidence and severity of arrhythmias was tested with contingency tables using the chi-squared test. The statistical significance level was $P < 0.05$.

## Results

The data obtained in the control experiments did not differ from those previously reported by our group and others [5–8]. Also, melatonin did not alter the action potential configuration, the ECG, the HR or the CF before coronary ligation. Fig. 1 illustrates typical results from experiments where the membrane potential was recorded synchronously with the ECG in a control heart (upper row) and in the presence of 5 $\mu$M (middle row) and 20 $\mu$M (lower row) melatonin. The records obtained with 10 and 50 $\mu$M melatonin are not shown because they were similar to those found with 20 $\mu$M. Coronary ligation produced a fast fall in RP in the three groups that reached its steady state within 3 min. This led to a concomitant decrease in the APA and the reduction of the overshoot (OS). There was a slow rising foot that preceded the fast depolarization particularly in records with 5 and 20 $\mu$M melatonin. These effects persisted throughout the ischemic insult. The APD decreased towards the end of ischemia in the control and the 5 $\mu$M melatonin hearts. By contrast, the hearts perfused with 20 $\mu$M melatonin showed an early increase in $APD_{90}$ followed by a progressive return to values to the

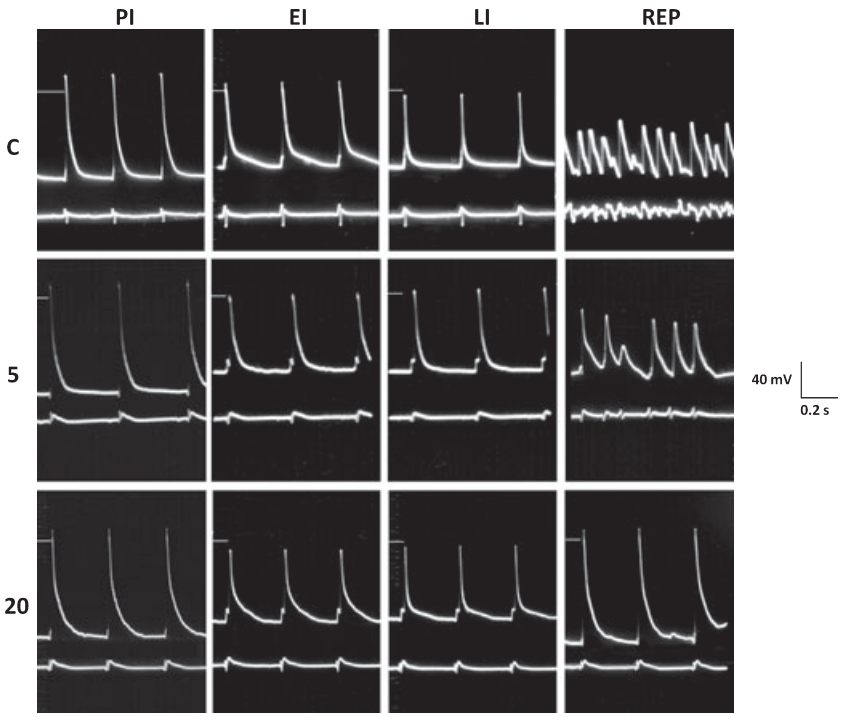


*Fig. 1.* Synchronous recordings of the transmembrane potential (upper traces) and the surface electrogram (lower traces) obtained at comparable times in a control heart (upper row) and in those exposed to 5 $\mu$M (middle row) or 20 $\mu$M melatonin (lower row). The columns correspond (from left to right) to preischemia (PI), early (EI) and late ischemia (LI) followed by reperfusion (REP), less than 1 min. Calibration, 40 mV and 0.2 sec. Horizontal white traces, 0 Volt reference level.

preischemic level. Reperfusion produced almost immediately a severe ventricular arrhythmia in the control hearts. A transient episode of tachycardia is shown with 5 $\mu$M melatonin in one of the hearts that developed ventricular tachycardia. Meanwhile, the heart with 20 $\mu$M melatonin recovered the preischemic morphology almost immediately.

Fig. 2A shows the quantitative analysis of the changes observed in the membrane potential of ventricular cells undergoing the ischemia-reperfusion protocol. As already mentioned, melatonin per se did not affect the resting or the APA in any of the experimental phases of the protocols. The early reduction of the APA, which persists during ischemia (see values of EI and LI), corresponds to the sum of the OS reduction plus the resting depolarization. The latter thus are mainly responsible for the changes in the electrical signal and its ability to propagate. The time course of the resting depolarization mimics that of the cellular $K^+$ accumulation during ischemia, in agreement with accepted knowledge [26, 27]. This result in a decreased availability of the $Na^+$ current system, OS disappearance and decreased effectiveness of the action potential to propagate, caused the slow rising foot that preceded the fast depolarization in our records (Fig. 1). Total recovery of the action potential characteristics was observed in all the hearts that maintained or recovered sinus rhythm during reperfusion.

The changes in $APD_{90}$ during the ischemia-reperfusion protocol in all groups are shown in Fig. 2B. During the preischemic period, melatonin did not affect $APD_{90}$ values; however at EI of all the hearts showed a lengthening that was significant for 10 and 50 $\mu$M melatonin. The latter group was also prolonged when compared with control for the same period. When analyze, the last period of ischemia versus the values of EI, a decrease in $APD_{90}$ was observed in all groups. Compared with preischemic results only control and 5 $\mu$M melatonin suffered a shortening; meanwhile the hearts exposed to 10, 20 and 50 $\mu$M melatonin reached levels similar to PI. The $APD_{90}$ in 20 and 50 $\mu$M treated hearts was significantly prolonged with respect to the control group for the same period. On reperfusion, the preischemic levels were recovered. The significance of these results are shown in the legend of Fig. 2.

Fig. 3 illustrates the protective effects of melatonin on the reperfusion arrhythmias. All the control hearts prematurely developed tachyarrhythmias on reperfusion; 54.5% were irreversible and only 5 out of 11 (45.5%) spontaneously returned to sinus rhythm after 3–6 min. With melatonin addition to the perfusate, the total incidence of tachyarrythmias fell to 50% with 5 and 10 $\mu$M melatonin, to 40% with 20 $\mu$M and to 30% with 50 $\mu$M melatonin. Moreover, a progressive reduction of irreversible arrhythmias was as follow: 40% for 5 $\mu$M, 30% for 10 $\mu$M, 20% for 20 $\mu$M and only one heart out of 10 (10%) developed an irreversible arrhythmia at the highest concentration studied, 50 $\mu$M. The chi-squared test revealed a significant decrease in the incidence of total and irreversible arrhythmias. The values were: $P < 0.05$ for 5 and 10 $\mu$M and $P < 0.01$ for 20 and 50 $\mu$M melatonin.

The values for the TAC expressed as Ae/L are presented in Fig. 4. The analysis performed on myocardial homogenates at the end of reperfusion period showed that the exposure to melatonin induced a substantial increase that was highly significant ($P < 0.001$) and dose independent within the concentration range used. Table 1 lists the values of HR and CF in the five experimental groups studied. Melatonin did not alter the HR either during PI or during ischemia. All the hearts showed a similar behavior: a sudden decrease of HR at the beginning of ischemia which

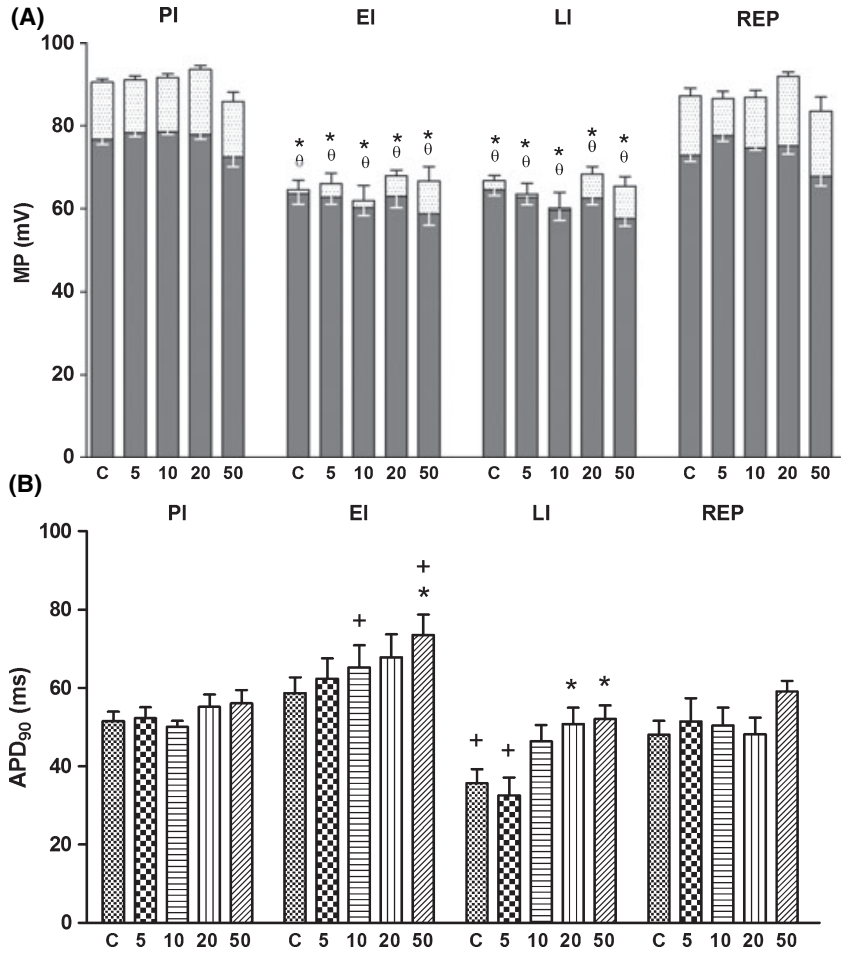


*Fig. 2.* Effects of the ischemia-reperfusion protocol on the membrane potential in control and melatonin-exposed hearts. (A) The bars represent the resting potential (darker shaded areas) and the action potential amplitude (total height) in control hearts (C) and in the presence of melatonin (concentration indicated under the abscissa). Each group illustrates data obtained before ischemia, preischemia (PI), at early (EI) and late ischemia (LI) and during reperfusion (REP). Means ± S.E.M., n = 11 for control and 10 in the others. During reperfusion, n corresponds to the number of hearts that did not suffer irreversible arrhythmias (see Fig. 3). $^{*}P < 0.001$ for APA and $^{\theta}P < 0.001$ for RP in EI and LI versus PI. (B) Changes in $APD_{90}$ with ischemia and reperfusion in control and melatonin exposed hearts, as indicated. Within each group, the bars represent means ± S.E.M. during (from left to right) PI, EI and LI followed by reperfusion in the hearts that recovered sinus rhythm or did not develop arrhythmias (see Fig. 3). $^{*}P < 0.05$ difference between control and melatonin groups; $^{+}P < 0.05$ for the PI values versus the values during ischemia for the same group.

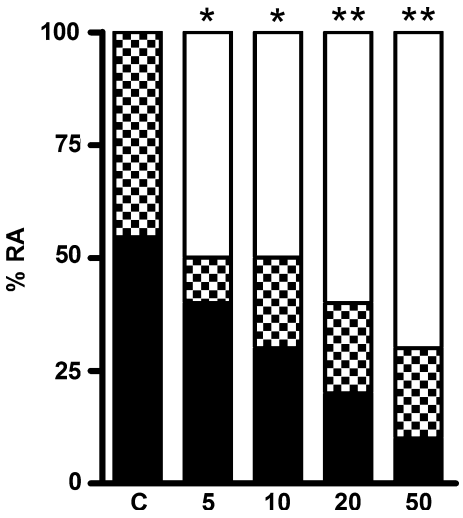


*Fig. 3.* Effects of melatonin on the incidence and severity of reperfusion arrhythmias. The black portions represent irreversible ventricular fibrillation (VF) and/or ventricular tachycardia (VT), the checkered portions illustrate VF and/or VT that spontaneously converted to sinus rhythm. The white portions correspond to absence of these ventricular arrhythmias. Control and melatonin concentrations in $\mu$M indicated under the abscissa. Chi-squared test for incidence, reversibility and absence of arrhythmia in melatonin exposed hearts versus control: $^{*}P < 0.05$, $^{**}P < 0.01$; n = 11 in control, 10 in the others.

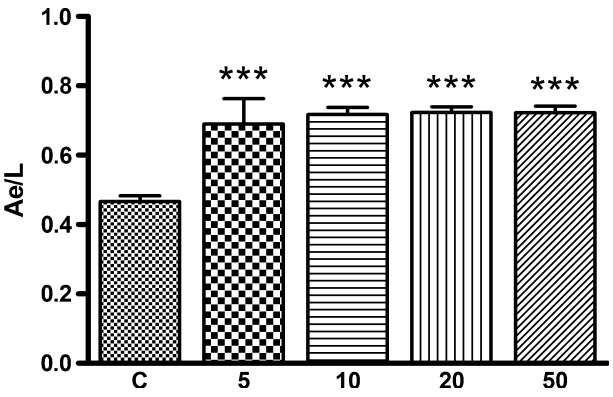


*Fig. 4.* Total antioxidant capacity determined on myocardial homogenates expressed as ascorbate equivalent per litre (Ae/L). C for control and numbers under the abscissa indicate melatonin concentrations in $\mu$M. C (n = 10); 5 (n = 5); 10 (n = 5); 20 (n = 7); 50 (n = 5). $^{***}P < 0.001$ when compared versus control group.

persists throughout, with a mean reduction of about 40 beats/min in all the groups; this is attributed to an endogenous acetylcholine release [28]. Although not significant from a statistical point of view, it was a constant observation. We attribute this lack of significance to the large S.E.M. that reflects the inherent instability of an isolated heart, deprived of autonomic control.

Table 1. Effect of melatonin on heart rate (HR) and coronary flow (CF) in the isolated perfused rat heart subjected to 10 min coronary occlusion and reperfusion

| | | HR (beats/min) | | CF (mL/min) | | |
|---|---|---|---|---|---|---|
| Group | n | Preischemia | Late ischemia | Preischemia | Ischemia | Reperfusion |
| C | 11 | 285.7 ± 9.5 | 249.7 ± 9.4 | 17.8 ± 1.4 | 7.8 ± 1.4* | 14.4 ± 2.0 |
| 5 | 10 | 263.7 ± 13.0 | 223.5 ± 10.2 | 13.6 ± 0.8 | 5.8 ± 0.6* | 10.3 ± 1.1 |
| 10 | 10 | 276.8 ± 14.0 | 236.7 ± 11.9 | 17.9 ± 1.5 | 7.5 ± 0.9* | 14.2 ± 1.6 |
| 20 | 10 | 272.1 ± 10.0 | 231.8 ± 12.4 | 20.2 ± 1.6 | 7.8 ± 0.6* | 15.4 ± 1.3 |
| 50 | 10 | 266.6 ± 15.5 | 215.0 ± 11.5 | 13.8 ± 1.6 | 8.2 ± 1.5* | 12 ± 1.7 |

Values are means ± S.E.M. of n hearts for each group. In group column, C = control and numbers correspond to melatonin concentration in $\mu$M. *$P < 0.05$ when compared with preischemia.

The CF was quite a stable variable considering the experimental model, the ischemic insult and the tachyarrhythmias triggered by reperfusion (Table 1). The significant reduction observed after ligation has also been reported by others [17, 21] and is currently used as criteria for the efficacy of the partial interruption of the coronary perfusion. Moreover, our records of the electrophysiological variables showed electrical characteristics compatible with cardiac ischemia. Reperfusion did not restore the flow to preischemic levels. It remained 3–5 mL/min below preischemic levels. This feature has also been reported by others and is referred as the no-reflow phenomenon [29].

## Discussion

The data presented above confirmed the strong antioxidant power of melatonin, in agreement with several reports in the literature [9–17]. Furthermore, our data showed that melatonin not only decreased the incidence of RA but also modified some characteristics of transmembrane potential. This effect has never been reported before, most probably because of the technical difficulties involved in intracellular recordings in an isolated rat heart beating at frequencies between 250 and 300 beats/min. In contrast to the antioxidant effects, clearly dose independent in our hands, our evidence shows, however, that the protection against RA was increased by administration of 20 and 50 $\mu$M melatonin.

While melatonin lacked of effect on the electrophysiological variables at PI, an analysis of the changes in $APD_{90}$ showed a lengthening at EI in relation with melatonin doses. Thereafter, all hearts suffered a progressive shortening that in LI reached pre-ischemic levels only in the 20 and 50 $\mu$M melatonin treated hearts.

Consequently, we propose that, in addition to the ionic imbalance and the presence of oxidant radicals as generators of arrhythmic activity on reperfusion, the role of the changes in electrical activity should not be overlooked, particularly considering the interaction of reactive oxygen species with ion transport mechanisms [30]. The APD is a key factor involved in the coordinated activation of the cardiac chambers and its alterations are involved in more than one arrhythmic activity. Even under regulation from the autonomic nervous system, APD is also the target of several antiarrhythmic treatments.

Amiloride derivatives and the increase in extracellular $Mg^{2+}$ concentration block the $K^+$ inwardly rectifying channel, and 4-aminopyridine blocks the transient outward current, all of them lengthen the action potential, and counteract RA [5–8].

Our experimental findings support the view that the protective effect of melatonin on the incidence of RA is related partially to its antioxidant action within the concentration range used in this study. The strong reduction in the incidence of arrhythmias in the melatonin-treated hearts confirms other data in the field [17–21]. In addition, at levels higher than 5 $\mu$M, melatonin counteracts the action potential shortening induced by ischemia thus exerting protection at another level in the chain of events that contribute to the development of RA. This effect could be independent of its antioxidant capacity or a modulation of the oxidative injury caused by the ischemia due to the accumulation of melatonin and/or its metabolites prior to the interruption of the CF [21].

In conclusion, these experiments confirm once more the importance of FR in the generation of the ionic imbalance and membrane instability related to the production of reperfusion arrhythmias. Moreover, they show that this ionic imbalance does not necessarily reflect irreversible cell injury, because arrhythmia disappears spontaneously in 50% of the cases when the ischemia is rather brief. In addition, the first event characteristic of myocardial ischemia is an increased $K^+$ permeability inducing cell $K^+$ loss with subsequent extracellular $K^+$ accumulation. This causes a rapid fall in the cell RP leading to a decrease in APA and slowing of conduction within 3 min. These phenomena create electrical inhomogeneity, a functional substrate for development of re-entry pathways. These changes are followed by variations of $APD_{90}$, a major determinant of refractory period. Our data show that 10, 20 and 50 $\mu$M melatonin modulates the changes in $APD_{90}$ acting in a similar way as $Mg^{2+}$ [6]. As a result, $APD_{90}$ lengthened at the beginning of the ischemia, then progressively shortened back to control levels before reperfusion, and remained there without further changes. The data add another characteristic to this versatile indolamine in the field of cardiovascular protection, specifically related to its electrophysiological properties. Further experiments in this field would seem highly worthwhile and support the rationale for the clinical trial proposed by Dominguez-Rodriguez et al. [22].

## Acknowledgments

The authors are grateful to Dr. Elena Ruiz Petrich for her critical reading and comments on the manuscript. This work was supported by grants 06/J177 from Secretaría de Ciencia, Técnica y Posgrado, Universidad Nacional de Cuyo and PIP 5192 from Consejo Nacional de Investigaciones Científicas y Técnicas.

## References

1. Zweier JL, Hassan Talukder MA. The role of oxidants and free radicals in reperfusion injury. Cardiovasc Res 2006; **70**:181–190.
2. Brooks WW, Conrad CH, Morgan JP. Reperfusion induced arrhythmias following ischemia in intact rat heart: role of intracellular calcium. Cardiovasc Res 1995; **29**:536–542.
3. Bernier M, Hearse DJ, Manning AS. Repefusion-induced arrhythmias and oxygen-derived free radicals. Studies with "anti-free radical" interventions and a free radical-generating system in the isolated perfused rat hearts. Circ Res 1986; **58**:331–340.
4. Keurs HDJ, Boyden PA. Calcium and arrhythmogenesis. Physiol Rev 2007; **87**:457–506.
5. Ruiz Petrich E, Ponce Zumino A, Moffat M, Rioux Y, Schanne O. Modulation of the electrophysiological effects of ischemia reperfusion by methylisobutyl amiloride. J Mol Cell Cardiol 1996; **28**:11–29.
6. Ponce Zumino AZ, Risler NR, Schanne O, Ruiz Petrich E, Carrión A. Magnesium: effects on reperfusion arrhythmias and membrane potential in isolated rat hearts. Mol Cell Biochem 1997; **171**:85–93.
7. Baiardi G, Ponce Zumino AZ, Ruiz Petrich E. Effects of barium and 5-hydroxydecanoate on the electrophysiologic response to acute regional ischemia and reperfusion in rat hearts. Mol Cell Biochem 2003; **254**:185–191.
8. Baiardi G, Carrión A, Ruiz Petrich E, Ponce Zumino AZ. 4-Aminopyridine: effects on electrical activity during ischemia and reperfusion in perfused rat hearts. Mol Cell Biochem 2002; **233**:85–90.
9. Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res 2007; **42**:28–42.
10. Maldonado MP, Murillo-Cabezas F, Terron MP et al. The potential of melatonin in reducing morbidity–mortality after craniocerebral trauma. J Pineal Res 2007; **42**:1–11.
11. Gilad E, Cuzzocrea S, Zingarelli B, Salzman AL, Szabo C. Melatonin is a scavenger of peroxynitrite. Life Sci 1997; **60**:L169–L174.
12. Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocr J 1993; **1**:57–60.
13. Pieri C, Marra M, Moroni F, Rechionni R, Marcheselli F. Melatonin: a peroxyl radical scavenger more effective than vitamin E. Life Sci 1994; **55**:PL271–PL276.
14. Macchi M, Bruce J. Human pineal physiology and functional significance of melatonin. Front Neuroendocrinol 2004; **25**:177–195.
15. Vanecek J. Cellular mechanisms of melatonin action. Physiol Rev 1998; **78**:687–721.
16. Reiter RJ, Tan DX. Melatonin: a novel protective agent against oxidative injury of the ischemic/reperfused heart. Cardiovasc Res 2003; **58**:10–19.
17. Tan DX, Manchester LC, Reiter RJ, Qi W, Kim SJ, El-Sokkary GH. Ischemia/reperfusion-induced arrhythmias in the isolated rat heart: prevention by melatonin. J Pineal Res 1998; **25**:184–191.
18. Bertuglia S, Reiter RJ. Melatonin reduces ventricular arrhythmias and preserves capillary perfusion during ischemia-reperfusion events in cardiomyopathic hamsters. J Pineal Res 2007; **42**:55–63.
19. Vazan R, Pancza D, Béder I, Styk J. Ischemia-reperfusion injury: antiarrhythmic effect of melatonin associated with reduced recovering of contractility. Gen Physiol Biophys 2005; **24**:355–359.
20. Kaneko S, Okumura K, Numaguchi Y et al. Melatonin scavenges hydroxyl radical and protects isolated rat hearts from ischemic reperfusion injury. Life Sci 2000; **67**:101–112.
21. Lagneux C, Joyeux M, Demenge P, Ribuot C, Godin-Ribuot D. Protective effects of melatonin against ischemia-reperfusion injury in the isolated rat heart. Life Sci 2000; **66**:503–509.
22. Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez MJ et al. A unicenter, randomized, double-blind, parallel-group, placebo-controlled study of Melatonin as an Adjunct in patients with acute myocaRdial Infarction undergoing primary Angioplasty The Melatonin Adjunct in the acute myocaRdial Infarction treated with Angioplasty (MARIA) trial: study design and rationale. Contemp Clin Trials 2007; **28**:532–539.
23. Tengattini S, Reiter RJ, Tan DX, Terron MP, Rodella LF, Rezzani R. Cardiovascular diseases: protective effects of melatonin. J Pineal Res 2008; **44**:16–25 Doi:10.1111/j.1600-079X.2007.00518.x.
24. Walker MJA, Curtis MJ, Hearse DH et al. The Lambeth Conventions: guidelines for the study of arrhythmias in ischemia, infarction and reperfusion. Cardiovasc Res 1988; **22**:447–455.
25. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999; **26**:1231–1237.
26. Kantor PR, Coetzee WA, Carmeliet EE, Dennis SC, Opie LH. Reduction of ischemic $K^+$ loss and arrhythmias in rat hearts. Effect of glibenclamide, a sulfonylurea. Circ Res 1990; **66**:478–485.
27. De Lorenzi F, Shufen C, Schanne OF et al. Partial contribution of the ATP-sensitive $K^+$ current to the effects of mild metabolic depression in rabbit myocardium. Mol Cell Bioch 1994; **132**:133–143.
28. Kawada T, Yamazaki T, Akiyama T et al. Differential acetylcholine release mechanisms in the ischemic and non-ischemic myocardium. J Mol Cell Cardiol 2000; **32**:405–414.
29. Reffelmann T, Kloner RA. The no-reflow phenomenon: A basic mechanism of myocardial ischemia and reperfusion. Basic Res Cardiol 2006; **101**:359–372.
30. Kourie JI. Interaction of reactive oxygen species with ion transport mechanisms. Am J Physiol 1998; **275** (Cell Physiol. 44):C1–C24.