**Effects of Melatonin on Ischemia and Reperfusion Injury of the Rat Heart**

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**Summary.** Effects of melatonin on various manifestations of ischemia/reperfusion injury of the isolated perfused rat heart were examined. Ischemia- and reperfusion-induced ventricular arrhythmias were studied under constant flow in hearts subjected to 10, 15 or 25 min of regional ischemia (induced by LAD coronary artery occlusion) and 10-min reperfusion. Melatonin was added to the perfusion medium 5 min before ischemia at concentrations of 10 µmol/l or 10 nmol/l and was present throughout the experiment. Recovery of the contractile function was evaluated under constant perfusion pressure after 20-min global ischemia followed by 40-min reperfusion. Hearts were treated with melatonin at a high concentration (10 µmol/l) either 5 min before ischemia only (M1) or 5 min before ischemia and during reperfusion (M2) or only during reperfusion (M3). At the high concentration, melatonin significantly reduced the incidence of reperfusion-induced ventricular fibrillation and decreased arrhythmia score (10% and 2.2 ± 0.3, respectively) as compared with the corresponding untreated group (62% and 4.1 ± 0.3, respectively); the low concentration had no effect. This substance did not affect the incidence and severity of ischemic arrhythmias. Melatonin (M2, M3) significantly improved the recovery of the contractile function as compared with the untreated group; this protection did not appear if melatonin was absent in the medium during reperfusion (M1). Our results show that melatonin, in accordance with its potent antioxidant properties, effectively protects the rat heart against injury associated with reperfusion. It appears unlikely that melatonin is cardioprotective at physiological concentrations.

**Key Words.** melatonin, heart, ischemia, reperfusion, arrhythmias, protection

**Introduction**

Melatonin (N-acetyl-5-methoxytryptamine), the hormone synthesized mainly in the pineal gland, regulates mammalian circadian and seasonal rhythms [1,2]. In humans, it has been often used for various indications, in particular for treatment of sleep disorders and relief of jet-lag symptoms [3,4]. In addition to its important role in the regulation of rhythms, melatonin has also been demonstrated to act as an immune system modulator [5] and efficient antioxidant and scavenger of reactive oxygen species [6,7]. This latter effect is considered to be involved in melatonin-induced attenuation of ischemia/reperfusion injury, as demonstrated in liver [8], brain [9,10], blood microvessels [11], and stomach [12].

Concerning the potential protective effects of melatonin in the heart, the available data are not consistent, although consensus exists that reactive oxygen species play an important role in the pathogenesis of cardiac reperfusion injury [13,14]. Thus, melatonin has been shown to increase cardiac electrical stability [15], reduce the incidence of reperfusion-induced ventricular arrhythmias [16–18] and to decrease the infarct size [17] in the isolated rat heart. In the same experimental model, either protective or no effect of melatonin was demonstrated on the post-ischemic recovery of contractile function and energy metabolism [18,19]. In addition, this substance did not influence the infarct size in the rabbit model of ischemia/reperfusion [20]. It is unclear what may be the cause of these differences and whether melatonin can be cardioprotective also at low physiological concentrations. Therefore, the goal of the present study was to examine the effects of two concentrations (10 µmol/l and 10 nmol/l) of melatonin on ischemia/reperfusion injury of the isolated perfused rat heart, manifested as ischemia- or reperfusion-induced ventricular arrhythmias and post-ischemic contractile dysfunction.

**Methods**

Adult male Wistar rats weighing 240 to 400 g were used. The investigations were performed in compliance with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

**Perfusion of the heart**

The rats were anesthetized with intraperitoneal sodium pentobarbital (60 mg/kg). Their hearts were...
rapidly excised, transferred to the perfusion apparatus and perfused by the Langendorff technique. Perfusion conditions differed, depending on the end point of the injury studied. For measurement of post-ischemic recovery of the contractile function, the hearts were perfused under constant pressure (100 cm H2O) with non-recirculating Krebs-Henseleit solution containing (mmol/l): NaCl 118, KCl 4.7, CaCl2 1.25, MgSO4 1.2, NaHCO3 25.0, KH2PO4 1.2, and glucose 7.0. The solution was saturated with 95% O2 and 5% CO2 (pH 7.4) and maintained at 37°C. The left ventricle was vented via a crease in the left atrium, and connected to the Hewlett-Packard (HP 1280) transducer. The balloon was gradually filled with water to give a diastolic pressure of 7.5 to 10 mm Hg. The amplified pressure signal was monitored and analyzed on a computer using our program. The left ventricular systolic (LVSP), diastolic (LVDp) and developed pressure (LVdP/dt)max were expressed as the means of ten cardiac cycles during a 2 s sampling period at selected time intervals. Coronary flow was measured by timed collection of coronary effluent.

For analysis of ischemic and reperfusion arrhythmias, the hearts were perfused under constant flow (adjusted to approximately 10 ml per min per g) with the same solution as above, except for lower KCl (3.2 mmol/l), higher CaCl2 (2.5 mmol/l) and added sodium pyruvate (2.0 mmol/l). The expected heart weights were calculated from regression equations established on the basis of previous data from our laboratory for heart weight to body weight ratio [21]. Epicardial electrograms were recorded with platinum electrodes attached to the right atrium and the apex of the heart. Mean perfusion pressure was measured in the aortic cannula by a HP 1280 transducer. Both perfusion pressure and electrograms were continually registered on a Hewlett-Packard (HP 770B) recorder; signals were stored in a computer and subsequently analyzed by our computer program. The heart rate was calculated from the electrograms.

**Experimental protocol**

**Contractile function study:** After 25 min of stabilization, the hearts were subjected to 20 min of global ischemia followed by 40 min of reperfusion. Global ischemia was induced by clamping the inflow aortic cannula. During ischemia, the hearts were bathed at 35°C in Krebs-Henseleit solution saturated with 95% N2 and 5% CO2 (pH 7.4). After abrupt restoration of flow, the functional parameters were recorded at 5-min intervals and their recovery was expressed as percentage of initial pre-ischemic values.

To investigate the effect of melatonin (Sigma) on the recovery of contractile function, three protocols were used. The hearts were either treated with a higher concentration (10 µmol/l) of melatonin starting 5 min before ischemia, whereas the reperfusion solution was melatonin-free (M1) or melatonin was present both 5 min before ischemia and during reperfusion (M2) or it was added only at the onset of reperfusion (M3). Melatonin was dissolved in ethanol and diluted by the perfusion solution. Control hearts in each group were treated in the corresponding way with a solution containing the solvent (0.1 mmol/l ethanol). For presentation, the controls to M1 and M2 were pooled, as they did not differ in any parameter.

**Arrhythmia study.** After a 25-min stabilization, regional no-flow ischemia was induced with a silk ligature tightened around the left anterior descending coronary artery as described earlier [22]. Coronary occlusion was considered successful if perfusion pressure increased immediately.

To investigate the effect of melatonin (10 µmol/l or 10 nmol/l) on reperfusion arrhythmias, the hearts were subjected to 10 or 15 min of ischemia followed by 10 min of reperfusion. The higher concentration of melatonin was used under conditions of both 10 and 15 min of ischemia, while the lower concentration was used in the latter protocol only. Perfusion with melatonin started 5 min before ischemia and continued throughout the experiment. Respective control groups received 0.1 mmol/l ethanol.

To investigate the effect of melatonin on ischemic arrhythmias, the hearts were subjected to 25 min of ischemia, followed by 10 min of reperfusion. The hearts were treated with either melatonin (10 µmol/l) or solvent as above.

Ventricular arrhythmias were assessed during the ischemic insult or during reperfusion according to the Lambeth Conventions [23]. Premature ventricular complexes (PVCs) occurring as singles, salvos or tachycardia (a run of 4 or more consecutive PVCs) were counted separately. The incidence and the number of episodes of ventricular tachycardia (VT) and fibrillation (VF) were also evaluated. VF lasting more than two minutes was considered as sustained. The severity of arrhythmias in each group was evaluated by an arrhythmia score according to the incidence of the most severe form of arrhythmia that occurred in each individual heart (hearts with single PVCs were given a score of 1, salvos 2, VT 3, reversible VF 4 and sustained VF 5).

**Statistics**

The results are expressed as mean ± SEM; non-Gaussian distributed variables are expressed as median and range. Differences in the number of PVCs and the number of episodes of ventricular tachycardia between the groups were compared by the Mann-Whitney U