Short Communication

Ischemia-Reperfusion Injury – Antiarrhythmic Effect of Melatonin Associated with Reduced Recovering of Contractility

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Abstract. The effect of melatonin on reperfusion arrhythmias and postischemic contractile dysfunction was studied in the isolated rat heart. 25 min global ischemia was induced and followed by 30 min of reperfusion. Melatonin (10 μmol/l) was present in the perfusion solution during the whole experiment. Experiment revealed protective effect of melatonin on reperfusion-induced arrhythmias – arrhythmia score was significantly lower as well as the total time of arrhythmias duration was significantly shorter in melatonin group than in controls. On the other hand, post-ischemic recovering of contractility was significantly reduced in melatonin group.

Key words: Melatonin — Ischemia — Reperfusion — Heart — Rat

Hormone melatonin (MLT), secreted mainly by the pineal gland, serves as regulator of circadian and seasonal rhythms in mammals. Melatoninergic receptors were detected in many tissues and organs, including the heart (Witt-Enderby et al. 2003). MLT is also efficient antioxidant and scavenger of reactive oxygen species (Tan et al. 2000), that can easily cross cell membranes because of its amphiphilic molecule (Shida et al. 1994; Costa et al. 1995). Effect of MLT on ischemia-reperfusion (IR) injury of the heart was subject of several studies. However, results of these studies are not quite consistent – not all of them confirmed expected protective effect of MLT on IR injury. In addition, our previous experiment focused on the heart subjected to calcium paradox (which is involved also in the IR injury), suggested the possibility of negative influence of MLT on recovering of contractility after ischemia (Važan et al. 2003). Concerning this we decided for an experiment, aimed at investigation of the effects of MLT on the post-ischemic recovering of myocardial contractility, as well as on the incidence, severity and duration of arrhythmias during the initial minutes of reperfusion.

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Isolated hearts of adult male Wistar rats (250–300 g) were perfused retrogradely with oxygenated Krebs-Henseleit (KH) solution at 37 ℃, pH 7.4 and a constant perfusion pressure 75 mmHg (Langendorff technique). After 30 min of stabilization, 25 min global normothermic ischemia was induced by turn off the KH flow into aortic canula. Reperfusion after ischemia lasted 30 min. ECG for detection of reperfusion arrhythmias was recorded during first 5 min of reperfusion. After this, hearts were stimulated at 300 beats/min and coronary flow (CF), left ventricular systolic pressure (LVSP) and left ventricular diastolic pressure (LVDP), $+\mathrm{d}P/\mathrm{d}t_{\max}$ (index of contractility) and $-\mathrm{d}P/\mathrm{d}t_{\max}$ (index of relaxation) were measured in 5-min intervals. Left ventricular developed pressure (LVDevP) was calculated as difference of LVSP and LVDP. All above mentioned variables were measured also at the end of stabilization (at 300 beats/min).

Two groups were studied. In MLT group ($n = 10$), melatonin (10 μmol/l) was present in the perfusion solution during the whole experiment. In controls ($n = 10$) solution contained no MLT. Arrhythmias were assessed according to the Lambeth Conventions (Walker et al. 1988). Their severity was expressed by arrhythmia score (AS). AS was given to each heart according to the incidence of the most severe arrhythmia that occurred in this heart. Score 1 – hearts with single premature ventricular complexes; Score 2 – salvos; Score 3 – ventricular tachycardia (VT, 4 or more consecutive premature ventricular complexes); Score 4 – reversible ventricular fibrillation (VF); Score 5 – sustained VF (SVF, lasting more than 2 min). Total time of VT+VF+SVF was also evaluated. Post-ischemic CF, LVSP, LVDP, LVDevP, $+\mathrm{d}P/\mathrm{d}t_{\max}$ and $-\mathrm{d}P/\mathrm{d}t_{\max}$ were expressed as percentage of their base-line pre-ischemic values, measured during the 30th min of stabilization.

Gaussian distributed variables were expressed as mean ± SEM, non-Gaussian as median and confidence interval (CI). Depending on distribution non-parametric Mann-Whitney U test or unpaired *t*-test was used. Difference was considered significant if $p < 0.05$.

No significant differences were observed between MLT group and controls at the end of stabilization (Table 1). During reperfusion, AS was significantly ($p < 0.05$) lower in MLT group (3 [CI 2.87 to 3.33] *versus* 4 [CI 3.33 to 4.67] in controls). Incidence of VT was 100 % in both groups, but incidence of VF was 10 % in MLT group and 20 % in controls. SVF was found in no MLT heart, but in 40 % of control

Table 1. Baseline (pre-ischemic) values – measured at the end of stabilization

	CF (ml/min)	LVDevP (mmHg)	$+\mathrm{d}P/\mathrm{d}t_{\max}$ (mmHg/s)	$-\mathrm{d}P/\mathrm{d}t_{\max}$ (mmHg/s)
cont	11.0 ± 0.6	93.65 ± 1.56	3342.2 ± 176.8	2023.2 ± 90.3
MLT	10.3 ± 0.57	97.67 ± 3.98	3659.8 ± 172.6	2052.7 ± 104.3

CF, coronary flow; LVDevP, left ventricular developed pressure; $+\mathrm{d}P/\mathrm{d}t_{\max}$, index of contractility; $-\mathrm{d}P/\mathrm{d}t_{\max}$, index of relaxation; cont, controls; MLT melatonin group. Values are mean ± SEM.

Table 2. Recovering of measured parametres during reperfusion after 25 min of global ischemia

		Minute of reperfusion				
		10th	15th	20th	25th	30th
LVDP	cont	3347.7 ± 684	2969.1 ± 653	2664.8 ± 632	2424.0 ± 612	2321.2 ± 587
(% of baseline)	MLT	5597.0 ± 713*	4995.8 ± 661*	4653.5 ± 639*	4332.2 ± 616*	4125.8 ± 596*
LVDevP	cont	39.3 ± 6.7	43.6 ± 8.2	51.3 ± 8.5	56.6 ± 7.7	58.0 ± 7.0
(% of baseline)	MLT	19.7 ± 2.0*	22.6 ± 2.3*	26.8 ± 3.1*	33.2 ± 3.9*	38.8 ± 4.8*
$+\mathrm{d}P/\mathrm{d}t_{\max}$	cont	40.4 ± 6.0	45.6 ± 7.7	54.4 ± 8.3	59.7 ± 7.2	61.5 ± 6.5
(% of baseline)	MLT	20.7 ± 1.8**	23.6 ± 2.1*	28.1 ± 3.0**	34.9 ± 3.8**	40.7 ± 4.3*
$-\mathrm{d}P/\mathrm{d}t_{\max}$	cont	37.3 ± 5.7	42.0 ± 7.8	47.9 ± 7.6	52.7 ± 6.9	53.3 ± 6.0
(% of baseline)	MLT	22.9 ± 1.8*	24.9 ± 2.4(*)	28.2 ± 2.9*	33.8 ± 3.5*	39.1 ± 4.1
CF	cont	106.9 ± 6.9	96.2 ± 5.1	92.3 ± 5.0	89.9 ± 4.6	89.9 ± 5.0
(% of baseline)	MLT	119.4 ± 8.0	104.0 ± 4.5	99.4 ± 4.4	97.3 ± 4.3	95.6 ± 4.6

LVDP, left ventricular diastolic pressure; LVDevP, left ventricular developed pressure; $+\mathrm{d}P/\mathrm{d}t_{\max}$, index of contractility; $-\mathrm{d}P/\mathrm{d}t_{\max}$, index of relaxation; CF, coronary flow; cont, controls; MLT, melatonin group. Values are mean ± SEM; (*) $p = 0.05$; * $p < 0.05$; ** $p < 0.01$.

hearts. Also total VT+VF+SVF time was significantly ($p < 0.01$) shorter in MLT group (14.5 ± 2.88 s *versus* 147.45 ± 34.16 s). On the other hand, recovering of LVDP, LVDevP, $+\mathrm{d}P/\mathrm{d}t_{\max}$ and $-\mathrm{d}P/\mathrm{d}t_{\max}$ during reperfusion was significantly better in controls and CF did not differ significantly (Table 2).

Our results demonstrated spectacular protective effect of MLT on reperfusion-induced arrhythmias, but also the reduced recovering of contractility and ability for relaxation in MLT group. Protective effect of MLT on reperfusion arrhythmias is in line with results of Tan et al. (1998), Kaneko et al. (2000), Lagneux et al. (2000) and Szarszoi et al. (2001). Reduced recovering of contractility in our MLT group is in line with the result of our previous experiment with hearts subjected to calcium paradox (which is also involved in the IR injury). In this experiment, hearts in the MLT group showed significantly decreased recovering of contractility in the phase of calcium repletion (Važan et al. 2003). Based on the fact, that free oxygen radicals contribute to high incidence of reperfusion-induced arrhythmias (Ravingerová et al. 1999), we assume that the protective effect of MLT on reperfusion arrhythmias can be attributed probably to the antioxidant properties of this hormone. Controversial negative effect of MLT on post-ischemic contractile dysfunction in our experiment could be attributed to its influence on microtubules. Non-specific direct biding of MLT to tubulin, that occurs when higher (10^{-5} mol/l) concentrations of this hormone are used (Benitez-King and Anton-Tay 1993), can lead to disruption of microtubules (Huerto-Delgadillo et al. 1994) and subsequently probably to deterioration of cardiac contractility, because similar disruption induced by microtubule disaggregator – colchicine was also associated with deterioration of cardiac function (Tenpaku et al. 1998).

In conclusion, hormone melatonin protects rat isolated hearts against reperfusion-induced ventricular arrhythmias, but in our experimental conditions it also reduces post-ischemic recovering of contractility. Learning the mechanisms of these two controversial effects requires further experiments.

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