Effect of Melatonin on the Isolated Heart in the Standard Perfusion Conditions and in the Conditions of Calcium Paradox

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Abstract. The effect of melatonin (MLT) on the isolated rat heart was studied using the standard perfusion conditions (Langendorff preparation) and model of calcium paradox (Ca^{2+}-paradox). Ca^{2+}-paradox was induced by 1 minute perfusion with Ca^{2+}-free Krebs-Henseleit (KH) solution and subsequent 20 minutes perfusion with a normal Ca^{2+}-containing KH solution. In MLT group, MLT (10 μmol/l) was in the perfusion solution throughout the experiment. In controls, there was no MLT. Variables: heart rate, coronary flow, systolic and diastolic pressure, $+\mathrm{d}P/\mathrm{d}t$ max (index of contractility) and $-\mathrm{d}P/\mathrm{d}t$ max (index of relaxation) were measured at the end of stabilization, i.e. after 30 minutes of standard perfusion and then in the 5^{th}, 10^{th}, 15^{th}, 20^{th} minute after perfusion with Ca^{2+}-free KH solution. Results: There was no difference between MLT group and controls in the standard perfusion conditions at the end of stabilization. After perfusion with Ca^{2+}-free KH solution, systolic-diastolic difference (in the 10^{th}, 15^{th}, 20^{th} minute), $+\mathrm{d}P/\mathrm{d}t$ max (in the 5^{th}, 10^{th}, 15^{th}, 20^{th} minute) and $-\mathrm{d}P/\mathrm{d}t$ max (in the 15^{th} minute) were significantly decreased in MLT group in comparison to controls.
Conclusion: Melatonin didn't influence rat isolated heart in standard perfusion conditions but it made the heart more susceptible to Ca^{2+}-paradox.

Key words: Melatonin — Calcium paradox — Calcium — Heart — Rat

Introduction

Melatonin (MLT) is the principal hormone secreted by the pineal gland with a circadian rhythm characterized by elevated blood levels during the night and very low levels during the light phase of the day. The most established role of MLT is to coordinate biological rhythms but in many experiments, it was also acting as a

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potent scavenger of toxic free radicals and antioxidant, exerting protective effect against various oxidative injuries of different organs (Reiter et al. 1997; Horáková et al. 2000; Tan et al. 2000; Štětinová et al. 2002).

Evidence functionally linking the pineal gland and the heart is obviously not plentiful, however, there are some reasons to believe that there may be some interactions between these two organs. It is well known that many aspects of cardiac physiology exhibit some degree of circadian rhythmicity. MLT binding sites were demonstrated in the heart of quail (Pang et al. 1996), chicken and duck (Pang et al. 1993), mouse (Drew et al. 2001) and indirectly (using MLT receptor antagonist) in isolated rat papillary muscle (Abete et al. 1997). They were also found in human coronary arteries (Ekmekcioglu et al. 2001a), where they had 24 h variation in the expression (Ekmekcioglu et al. 2001b). MLT provided protection against adriamycin-induced cardiomyopathy in rats (Morishima et al. 1998) and against consequences of ischaemia-reperfusion injury of the rat heart (Tan et al. 1998; Lagneux et al. 2000; Salie et al. 2001; Szarszoi et al. 2001). It can also influence mechanisms involved in the calcium homeostasis of cardiomyocytes. MLT significantly increased the amplitude of the L-type (high-voltage) calcium current in the chicken embryonic heart cells (Mei et al. 2001), stimulated Ca^{2+}-pump in the sarcolemma of rat cardiomyocytes (Chen et al. 1993) and significantly decreased density of voltage-sensitive calcium channels in the rat cardiac membranes (Chen et al. 1994). In the hearts of cardiomyopatic hamsters, with defect in calcium efflux, MLT supported pathophysiological processes that led to decreased longevity of these animals (Natelson et al. 1997).

In the present study we tested the effect of MLT on the isolated rat heart in the standard perfusion conditions and in the conditions of calcium paradox (Ca^{2+}-paradox).

Materials and Methods

Animals

The experiments were performed on adult male Wistar rats (250–300 g body weight), fed on standard pellet diet and tap water ad libitum. Their endogenic production of MLT was inhibited by exposition to constant light (1500 Lux *per* cage) – 24 hours before experiments. Constant light abolished noctural rise of MLT levels in Wistar rats (Brown et al. 1991). By this manipulation we also tried to induce "hunger" of their tissues for MLT.

Study was performed in accordance with the Guide for the care and use of laboratory animals published by the US National Institute of Health (NIH publication No. 85–23, revised 1985).

Experimental protocols

The animals were anaesthetized with pentobarbitone sodium (60 mg/kg b.w. i.p.) and treated with heparin (500 IU i.p.) before excision of the heart. The excised

hearts were then perfused retrogradely with oxygenated Krebs-Henseleit (KH) solution at 37°C and a constant perfusion pressure 75 mm Hg (Langendorff preparation). KH solution (pH 7.4), gassed with 95 % oxygen and 5 % carboxide, contained in g/l: 2.0 glucose, 0.178 $CaCl_2$, 6.895 NaCl, 2.285 $NaHCO_3$, 0.295 $MgSO_4$, 0.136 KH_2PO_4, 0.224 KCl (filtered through – AE99-membrane filter, 8 μm). A water-filled latex balloon, coupled to a pressure transducer, was inserted into the left ventricular cavity *via* the left atrium. For epicardial electrogram recording an electrode was attached to the apex of the heart.

After 30 minutes of stabilization, Ca^{2+}-paradox was induced by 1 minute perfusion with Ca^{2+}-free KH solution (enriched with 0.1 mmol/l EDTA), followed by 20 minutes perfusion with a normal Ca^{2+}-containing KH solution.

Measured variables: heart rate (HR), coronary flow (CF), systolic (S) and diastolic (D) pressure, $+dP/dt$ max (index of contractility) and $-dP/dt$ max (index of relaxation). All variables were measured at the end of stabilization, i.e. after 30 minutes of standard perfusion and then in the 5^{th}, 10^{th}, 15^{th} and 20^{th} minute after perfusion with Ca^{2+}-free KH solution.

Experimental groups

Two groups were studied. In MLT group ($n = 11$), melatonin (10 μmol/l) was in the perfusion solution throughout the experiment. In controls ($n = 8$) solution contained no MLT.

Statistical analysis

The data (for each heart) obtained during the Ca^{2+}-paradox were transformed into the form of percentual deviation (Δ %) from initial data, measured at the end of stabilization. Results are presented as medians and their confidency intervals (CI). Statistical comparisons of groups were done by non-parametric Mann-Whitney U test. Difference was considered significant if $p < 0.05$.

Results

No significant differences in HR, CF, S-D difference, $+dP/dt$ max and $-dP/dt$ max were observed between MLT group and controls at the end of stabilization (Tab. 1). It means there was no effect of MLT on isolated rat heart in the standard perfusion conditions.

Several significant differences were observed after perfusion with Ca^{2+}-free KH solution. S-D difference was significantly decreased in MLT group in comparison to controls in the 10^{th} ($p = 0.02$), 15^{th} ($p = 0.02$) and 20^{th} ($p = 0.04$) minute (Fig. 1, Tab. 2). $+dP/dt$ max was significantly decreased in MLT group in comparison to controls in the 5^{th} ($p = 0.04$), 10^{th} ($p = 0.02$), 15^{th} ($p = 0.02$) and 20^{th} ($p = 0.03$) minute (Fig. 2, Tab. 2). $-dP/dt$ max was also significantly decreased in MLT group in comparison to controls but only in the 15^{th} ($p = 0.03$) minute. Decrease of $-dP/dt$ max in MLT group that was of borderline significance was measured in the 10^{th} ($p = 0.05$) minute (Fig. 3, Tab. 2). The above mentioned

Table 1. Effect of MLT on heart function in the standard perfusion conditions (measured at the end of stabilization)

End of stabilization	median		CI		
	controls	MLT	controls	MLT	
HR [beats/min]	240	248	224 to 295	215 to 281	$p = 0.84$
CF [ml/min]	7.8	7	6 to 9.6	6 to 8.4	$p = 0.49$
S-D [mm Hg]	93.5	88	50 to 105	70 to 135	$p = 0.66$
$+\mathrm{d}P/\mathrm{d}t$max [mm Hg/s]	3407.5	3350	1933 to 3878	1957 to 4583	$p = 0.60$
$-\mathrm{d}P/\mathrm{d}t$max [mm Hg/s]	−1571.5	−1687	−1925 to −929	−2433 to −1086	$p = 0.35$

HR, heart rate; CF, coronary flow; S-D, difference of systolic and diastolic pressure; +dP/dtmax, index of contractility; -dP/dtmax, index of relaxation. Data are medians and confidency intervals (CI). Statistical comparisons – Mann-Whitney U test.

results indicate that contractility and ability for relaxation were reduced in MLT group during Ca^{2+}-paradox. CF was evidently but insignificantly increased in MLT group in comparison to controls in the 10^{th}, 15^{th} and 20^{th} minute (Fig. 4, Tab. 2) and HR didn't show any significant difference between MLT group and controls (Fig. 5, Tab. 2) in the conditions of Ca^{2+}-paradox.

Discussion

Perfusion of the heart with calcium free solution (calcium depletion) and subsequently with normal calcium containing solution (calcium repletion) induces significant deterioration of myocardial functions with irreversible damage of the metabolism and structure of cardiomyocytes. This phenomenon is known as Ca^{2+}-paradox (Zimmerman and Hullsmann 1966). Crucial factor in its pathogenesis is intracellular overaccumulation of calcium with subsequent stimulation of energy utilizing processes and decrease in mitochondrial energy production (Hearse et al. 1978a; Duan and Karmazyn 1989). Opinions on mechanisms enabling increased influx of calcium in the phase of repletion differ: Chapman considers as a crucial factor an increase in intracellular natrium (in the phase of calcium depletion) with subsequent stimulation of Na^{+}-Ca^{2+} exchanger (Chapman 1987), Ganote reports, that the most important are ultrastructural changes in sarcoplasmatic membrane (Ganote 1991). The final result of Ca^{2+}-paradox is total lost of mechanical and electrical activity of the heart (Hearse et al. 1978b; Dhalla et al. 1983; Fleckenstein et al. 1983). Ca^{2+}-paradox differs from simple calcium overload by stronger injury of sarcoplasmatic membrane (Altschuld et al. 1991; Tribulova et al. 1993).

Interesting result of our study is the negative influence of MLT on resistance of isolated heart to Ca^{2+}-paradox. We used mild Ca^{2+}-paradox (with only 1 minute of calcium depletion), because results of different studies on possible effect of MLT in calcium homeostasis are not consistent. There was the same possibility for positive

Table 2. Effect of MLT on heart function in the conditions of Ca^{2+}-paradox (measured during Ca^{2+}-repletion phase – in the 5th, 10th, 15th, 20th minute)

Ca^{2+}-paradox	median		CI		
HR [Δ%]	controls	MLT	controls	MLT	
5	−2.909	−7.907	−16.45 to 6.58	−27.42 to 6.84	$p = 0.27$
10	−1.401	−1.179	−18.182 to 13.92	−8.05 to 6.46	$p = 0.83$
15	−3.147	−1.091	−10.4 to 15.61	−4.21 to 13.92	$p = 0.46$
20	−1.44	−1.464	−14 to 9.7	−4.84 to 18.14	$p = 0.46$
CF [Δ%]	controls	MLT	controls	MLT	
5	13.194	17.647	0 to 22.73	0 to 34.29	$p = 0.49$
10	2.708	15.966	−11.76 to 34.09	6.67 to 31.25	$p = 0.07$
15	2.083	19.538	−8.82 to 47.73	6.67 to 38.09	$p = 0.07$
20	5.417	18.609	−6.67 to 43.18	0 to 38.09	$p = 0.10$
S-D [Δ%]	controls	MLT	controls	MLT	
5	9.43	−40.26	−65.71 to 60	−77.89 to −7.143	$p = 0.06$
10	−5.479	−50.98	−67.62 to 36.1	−95.39 to −20	$p = 0.02$
15	−2.658	−52.273	−69.52 to 32.21	−96.71 to −22.86	$p = 0.02$
20	−2.658	−53.409	−68.13 to 18	−96.71 to −22.86	$p = 0.04$
$+dP/dt$max [Δ%]	controls	MLT	controls	MLT	
5	7.591	−36.573	−72.5 to 47.44	−83.76 to −20.98	$p = 0.04$
10	−5.672	−61.223	−74.43 to 25.66	−93.45 to −30.41	$p = 0.02$
15	−2.165	−59.608	−75.65 to 22.45	−94.11 to −32.28	$p = 0.02$
20	−1.05	−58.209	−74.77 to 9.52	−94.11 to −29.82	$p = 0.03$
$-dP/dt$max [Δ%]	controls	MLT	controls	MLT	
5	6.253	−33.568	−63.64 to 48.23	−75.44 to −3.64	$p = 0.07$
10	−4.805	−48.31	−66.69 to 22.51	−91.2 to −9.09	$p = 0.05$
15	0.579	−50.944	−66.69 to 17.9	−91.37 to −18.18	$p = 0.03$
20	2.651	−49.602	−64.81 to 13.37	−91.37 to −9.1	$p = 0.06$

HR, heart rate; CF, coronary flow; S-D, difference of systolic and diastolic pressure; $+dP/dt$max, index of contractility; $-dP/dt$max, index of relaxation. Data are medians of percentual deviations from baseline data measured at the end of stabilization. CI, confidency interval. Statistical comparisons – Mann-Whitney U test.

as for negative influence of MLT. It means that longer calcium depletion would overlap the possible negative effect of MLT by too strong injury of the heart.

Chen et al. published a study about stimulatory effect of MLT on Ca^{2+}-pump in the rat cardiac sarcolemma (Chen et al. 1993) and another study in which MLT significantly decreased density of cardiac voltage-sensitive calcium channels in rats (Chen et al. 1994). This should indicate for possible protective effect of MLT. On the contrary, Mei et al. (2001) published a study in which MLT increased L-type high-voltage channels calcium influx in chicken embryonic heart cells. Also

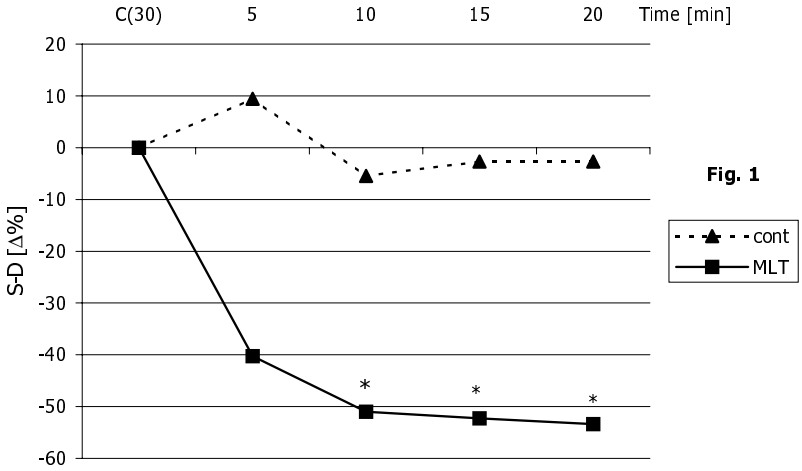


Fig. 1

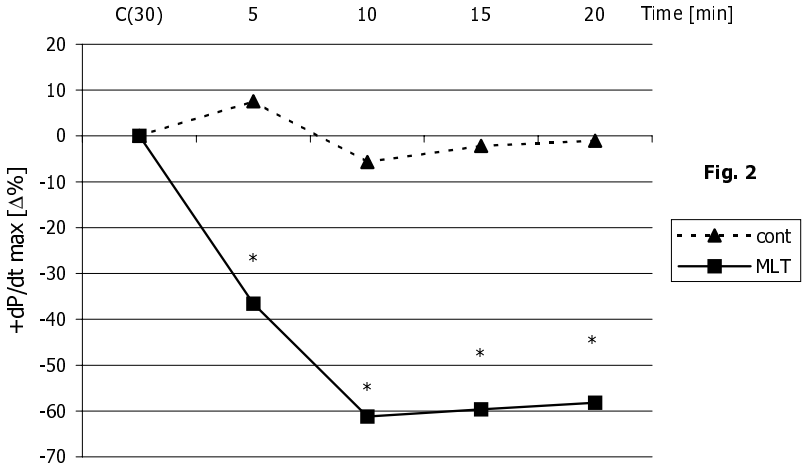


Fig. 2

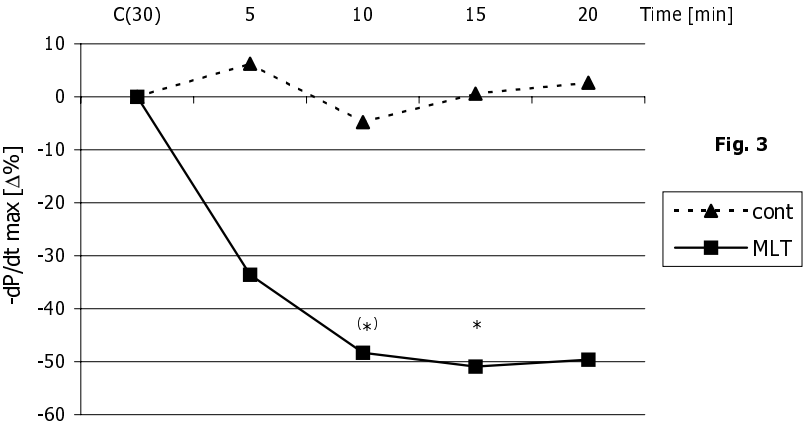
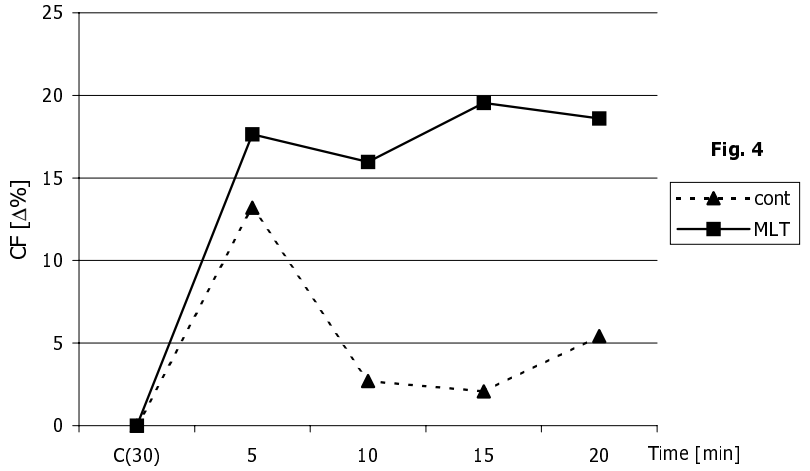


Fig. 3



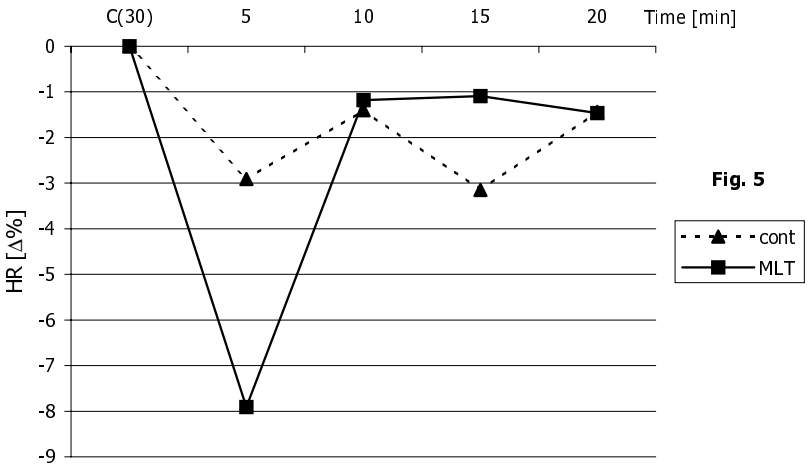


Figure 1–5. Effect of MLT on systolic-diastolic difference (S-D), contractility ($+\mathrm{d}P/\mathrm{d}t$ max), relaxation ($-\mathrm{d}P/\mathrm{d}t$ max), coronary flow (CF) and heart rate (HR) in the conditions of Ca^{2+}-paradox – measured during Ca^{2+}-repletion phase (in the 5^{th}, 10^{th}, 15^{th}, 20^{th} minute). Results are displayed as medians of percentual deviations from baseline data measured at the end of stabilization. C(30) – end of stabilization (control). Statistical comparisons of MLT group and controls were done by Mann-Whitney U test. * $p < 0.05$, $^{(*)}$ $p = 0.05$.

study of Natelson et al. (1997) with cardiomyopathic hamsters indicates negative influence of MLT on the development of cardiomyopathy. It is known that the hearts of cardiomyopathic hamsters show problematic calcium efflux and MLT probably aggravates this defect. Our results support hypothesis on possible negative effect of MLT on calcium homeostasis of the heart in some special conditions.

The above mentioned studies indicate that negative influence of MLT in our study might be a consequence of an increased calcium influx, but with the same probability it could also be a consequence of a decreased calcium efflux. Elucidation of involved mechanisms will require further studies.

Our study also shows that MLT didn't influence any of the measured variables of the isolated heart in standard perfusion conditions. Similarly, in the study of Abete et al. (1997) MLT didn't show any direct inotropic effect on isolated papillary muscle of the rat heart.

In conclusion, MLT didn't influence rat isolated heart in the standard perfusion conditions but it made the heart more susceptible to myocardial injury mediated by Ca^{2+}-paradox. Learning the mechanisms of this effect requires further research.

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