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Cardioprotective effect of melatonin against ischaemia/reperfusion damage

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1. ABSTRACT

Melatonin (N-acetyl-5-methoxytryptamine) has been shown by several workers to protect the heart against ischaemia/reperfusion damage. Melatonin, both in the picomolar and micromolar range, significantly reduces infarct size and improves functional recovery during reperfusion. This may be due to its free radical scavenging and anti-oxidant effects, while the melatonin receptor and its marked anti-adrenergic actions may also be involved. The latter is mediated by nitric oxide (NO), guanylyl cyclase and protein kinase C (PKC). Melatonin-induced cardioprotection is associated with activation of protein kinase B (PKB), extracellular signal-regulated kinase (ERK1/2) (the Reperfusion Injury Salvage Kinase (RISK) pathway) and signal activator and transducer 3 (STAT-3) (the Survivor Activating Factor Enhancement (SAFE) pathway) during reperfusion and inhibition of the mitochondrial permeability transition pore (MPTP). Very little is known about the effect of melatonin on myocardial substrate metabolism. Melatonin was demonstrated to be involved in the regulation of whole body glucose homeostasis via its effects on pancreatic insulin secretion and may thus indirectly affect myocardial substrate metabolism in a circadian manner.

2. INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is a highly conserved molecule found in organisms from unicells to vertebrates (1). Produced by the pineal gland, it has pleiotropic bioactivities which include, amongst others, several endocrinological processes (for a review see ref 2). The finding by Tan *et al* (3) that melatonin is a potent free radical scavenger and an anti-oxidant, has elicited enormous interest, stimulated considerable research efforts and several reviews have appeared on this topic (4-6). Since the major metabolites of melatonin, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N-acetyl-5-methoxykynuramine (AMK) also have free radical scavenging abilities, one molecule of melatonin can scavenge up to 10 reactive oxygen (ROS) or nitrogen (RNS) species (4).

Melatonin has beneficial effects in a surprising number of pathophysiological conditions purportedly associated with increased oxidative stress such as cardiovascular disease (7), Alzheimer's disease (8), diabetes (9), drug-mediated ototoxicity (10), to name but a few. In addition, melatonin has marked effects on the ageing process: its production is known to diminish in

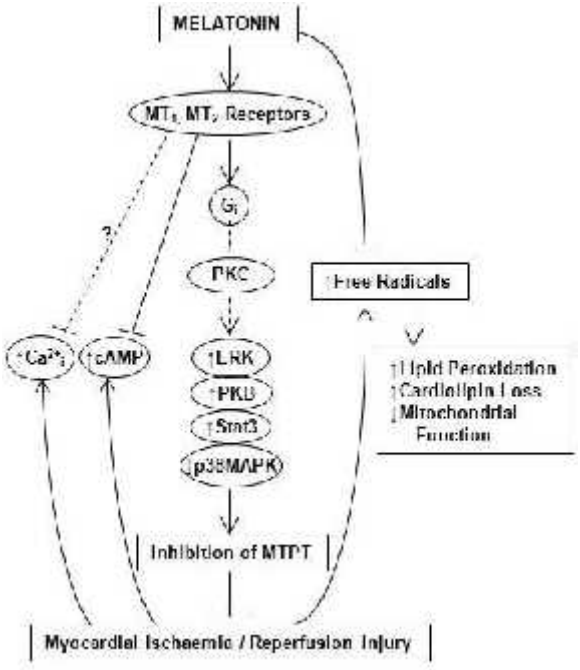

Figure 1. Effects of melatonin on myocardial ischaemia;/reperfusion injury. It may act via (i) scavenging of free radicals produced during I/R, thereby attenuating lipid peroxidation, mitochondrial cardiolipin loss and the reduction in mitochondrial function and/or via its receptors by (ii) attenuating the stimulation of the beta-adrenergic pathway during ischaemia and (iii) activation of the RISK and SAFE pathways, leading to inhibition of the mitochondrial permeability transition pore (MPTP).

elderly persons (11) and it is suggested to play a role in the elevated oxidative damage observed in this population (for a review see ref 12). Although the ageing process is complex and multifactorial, melatonin and its metabolites have multiple effects that may be beneficial for an ageing individual. It has recently been proposed that melatonin acts via increased expression of SIRT1, which reduces inflammatory and apoptotic signaling related to p53, increases NO bioavailability, vasodilatation and down regulates oxidative stress (13).

Melatonin has significant beneficial effects in several cardiovascular conditions such as hypertension, myocardial hypertrophy and ischaemia/reperfusion (I/R) injury (14). This review will focus mainly on the effects of melatonin on I/R injury as well as on its effects on myocardial substrate metabolism.

3. MELATONIN AND THE HEART

Under normal conditions melatonin treatment has no effects on heart function (15, 16), but long-term melatonin consumption reduces the absolute and relative heart weights (16,17) and increases its glycogen content (18). Melatonin also has beneficial effects on the heart in physiological conditions such as ageing: Petrosillo and coworkers (19) showed that melatonin administration to aged rats counteracted the increased susceptibility to Ca^{2+}-induced mitochondrial permeability transition pore opening associated with increased cytochrome C release as well as cardiolipin oxidation/depletion. In addition, it improves other pathophysiological conditions, for example hyperthyroidism (20), cadmium-induced oxidative damage (21) and myocardial hypertrophy (22).

Pinealectomy has profound effects on the myocardium: not only does it increase its susceptibility to I/R damage (see below), but it affects the heart by causing increased serum cholesterol and cardiac malondialdehyde levels, as well as heart weight. Furthermore, hearts from pinealectomized animals exhibited increased myocardial fibrosis, myxomatous degeneration of the valves and thickening of the left atrial endocardium (23).

A further link between melatonin and the heart in pathophysiological conditions is the fact that nocturnal melatonin has been reported to be decreased in patients with coronary heart disease (24) or acute myocardial infarction (25). In addition, the observed circadian rhythm of sudden death caused by heart disease correlates inversely with the rhythm of circulating melatonin (26,27). Interestingly, the rate of sudden cardiac death is highest in the early morning hours when plasma melatonin levels are at their lowest.

4. MELATONIN AND ISCHAEMIA/REPERFUSION INJURY

In view of the pivotal role of generation of free radicals in I/R injury, the possible beneficial effects of melatonin on the ischaemic reperfused heart have received much attention during the past few years, as evidenced by a number of recent reviews (7,28-30).

Most of the studies thus far employed the rat as experimental animal, using pharmacological concentrations of the hormone. Briefly, using the isolated perfused heart as model, melatonin was shown to reduce premature ventricular contractions and fibrillation (31-33), improve functional recovery during reperfusion (29-32) and to reduce infarct size (34-37). Melatonin-induced cardioprotection was found to be associated with suppression of myeloperoxidase activity (MPO) (38-40) and malondialdehyde (MDA) levels (38, 40), suggesting a reduction in lipid peroxidation (Figure 1).

In view of the generation of large amounts of free radicals at the onset of reperfusion (41), it is important that melatonin is present in the heart at the time of reperfusion: thus it has to be administered both before and after ischaemia or during reperfusion only to induce cardioprotection *in vitro* (35). However, melatonin administered *in vivo* before the ischaemic insult, was also effective (39). It is assumed that, under these circumstances, melatonin was still present in sufficient quantities at the onset of reperfusion to induce effective protection (39). Interestingly, melatonin given in a preconditioning mode (brief administration followed by washout before onset of ischaemia), elicits cardioprotection

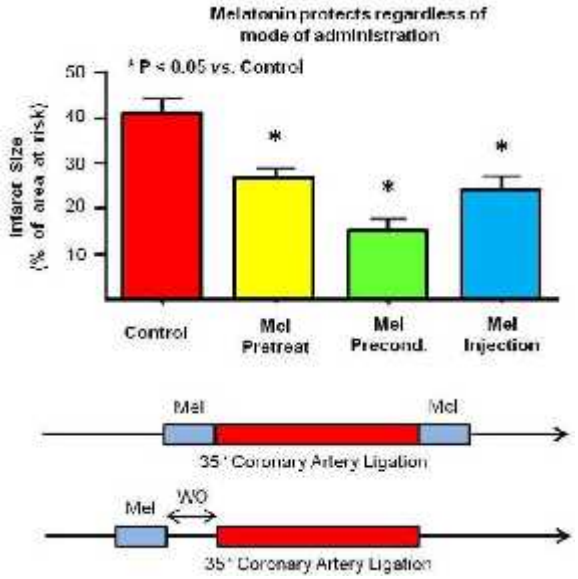

Figure 2. Melatonin treatment reduced infarct size whether administered in the perfusate (50microM) for (i) 10 min before and after exposure of the heart to 35 min coronary artery ligation (pretreatment) or (ii) before ischaemia followed by 10 min reperfusion (preconditioning mode) or injected intraperitoneally (5mg/kg) before perfusion in the absence of melatonin (n=6/group) (Lochner et al, unpublished data). *P<0.05 vs control.

(432) (See Figure 2). Melatonin is capable of producing long-term cardioprotection since intraperitoneal administration 24h prior to experimentation or oral administration for several days followed by subsequent perfusion in the *absence* of the hormone, also caused a reduction in infarct size (Lochner, unpublished data). We concluded from these studies that melatonin is indeed a powerful protectant against ischaemic damage.

A wide range of pharmacological concentrations of melatonin (in the micromolar range) was used in most of the above studies (33-35). However, a recent report showed that reduction of endogenous circulating melatonin by pinealectomy, exacerbated myocardial injury as indicated by a larger infarct size and an increased mortality resulting from irreversible ventricular fibrillation (43,44). These findings suggested that the relatively low endogenous melatonin levels normally present in the circulation, are effective in reducing I/R-induced myocyte damage and it was suggested by these workers that pharmacological concentrations of melatonin did not add to its beneficial actions (43,44). A recent study by Lamont and coworkers (42) also found melatonin in the picomolar range to be protective in retrogradely perfused rat hearts. However, using the isolated perfused working rat heart, it was shown that melatonin was effective in the micromolar range only (35). As far as we know, the effects of melatonin, at concentrations in the picomolar range, have not been studied yet in the working rat heart model. It is possible that melatonin has a biphasic effect in these *in vitro* perfused hearts, but this remains to be determined.

5. MELATONIN SIGNALLING

5.1. Role of melatonin receptors

It is generally accepted that melatonin exerts its cardioprotective actions via its free radical scavenging activities as well as the induction of anti-oxidant enzymes (45-47). Convincing evidence also exists for a role for the melatonin receptors in this regard (see below).

In mammals, melatonin signals through activation of at least two high-affinity G protein-coupled receptors, MT_1 and MT_2 (for review see ref 48). The MT_1 receptor has been identified in chicken (49) and human (50) coronary arteries as well as in chicken (51) and rat hearts (52), coronary arteries and aorta (50). As far as we know, the presence of the MT_3 receptor has not yet been demonstrated in heart muscle. The significance of the melatonin receptor in melatonin-induced cardioprotection was convincingly demonstrated by the finding that luzindole, a non-selective melatonin receptor antagonist, abolished its cardioprotective actions, using infarct size as endpoint (35).

Most, if not all, studies on the interactions between melatonin and the heart, focussed on the effects of exogenous melatonin on the myocardial response to I/R. In contrast, a recent study investigated the effects of myocardial infarction on the synthesis, concentration and receptor expression of endogenous melatonin. Sallinen and coworkers (53) showed that induction of myocardial infarction in rats caused a significant increase in left ventricular and plasma melatonin levels within 1 day, followed by a significant (2.8 fold) increase in MT_1 mRNA levels after 14 days, suggesting that melatonin is an important endogenous protector against I/R injury.

5.2. Intracellular signalling

The MT_1 and MT_2 receptors signal by coupling to heterotrimeric G_i proteins and downstream effector mechanisms which include adenylyl cyclase, PKC, phospholipase C, phospholipase A2, potassium channels, guanylyl cyclase and calcium channels (for reviews see 29,48,54). Melatonin regulates coronary vasomotor tone via the MT_2 receptor and stimulation of PDE5, which in turn, increases degradation of cGMP (55). Melatonin is also known to reduce cAMP accumulation in most tissues (54).

5.3. Anti-adrenergic effects

It is well-established that activation of the adrenergic nervous system and generation of cAMP play an important role in the genesis of dysrhythmias during reperfusion of the ischaemic heart (56). Since several workers have reported that melatonin reduces the incidence of ventricular fibrillation and arrhythmias during reperfusion of isolated rat hearts (31,33,34), it is possible that this may be due to its anti-adrenergic actions, as demonstrated by its effects on the contractility of isolated papillary muscles (52,57). This phenomenon was further investigated by determining the effects of melatonin on isoproterenol-induced beta-adrenergic stimulation. Genade

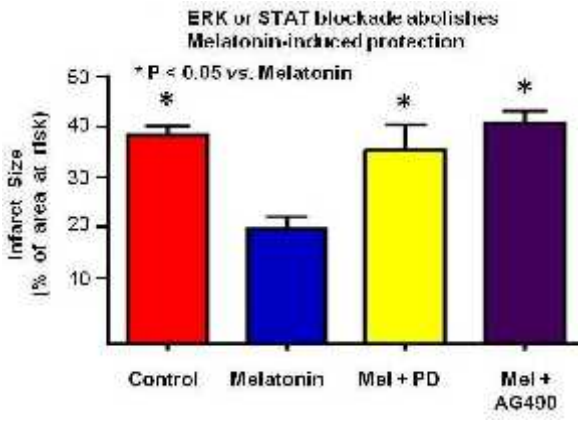

Figure 3. Melatonin-induced cardioprotection is ERK as well as STAT-3 dependent. Hearts were perfused with melatonin (50microM) with or without PD98059 (10microM) or AG-490 (5 microM) administered before and after 35 min regional ischaemia (n=6/

and coworkers (58) showed that melatonin significantly counteracted the powerful beta-adrenergic stimulation by both forskolin and isoproterenol in a receptor-dependent manner as indicated by tissue cAMP levels in normoxic perfused hearts. Nitric oxide (NO), guanylyl cyclase as well as PKC were involved in these potent anti-adrenergic actions of melatonin, since the inhibitors L-NAME, ODQ and bisindolyl-maleimide respectively, significantly counteracted these actions. As discussed above, melatonin activates PKC in a number of cell types (59,60). Should this also occur in heart muscle, it could be responsible for counteracting isoproterenol-induced cAMP generation via inhibitory cross-talk between PKC and PKA (61,62). These results suggest its anti-adrenergic actions may play a role in melatonin-induced cardioprotection, with a pivotal role for NO. A role for NO in the melatonin-induced preservation of liver function and structure during ischaemia/reperfusion has also been reported (63).

It is known that melatonin can protect the heart against beta-adrenergic (isoproterenol)-induced oxidative stress by restoring the activities and levels of anti-oxidant enzymes and by reducing free radical generation (64). Using the model of isoproterenol-induced myocardial infarction, Patel and coworkers (15) demonstrated that melatonin pretreatment reduced myocardial total cholesterol levels and increased phospholipids, compared to isoproterenol treatment alone. Whether melatonin has similar actions in the scenario of I/R remains to be established.

6. EVENTS DOWNSTREAM OF MELATONIN RECEPTOR STIMULATION

6.1. RISK and SAFE pathways

It was recently shown that the Reperfusion Injury Salvage Kinase (RISK) pathway, which includes the survival kinases protein kinase B (PKB)/Akt and extracellular signal-regulated kinase (ERK) 1/2, is associated with powerful cardioprotection when activated at the onset of reperfusion (for review see ref 65). In addition to this signalling pathway, it is also known that the cytokine tumor necrosis factor alpha (TNF-alpha) contributes to myocardial adaptation and plays a role in the protection conferred by ischaemic pre- and postconditioning (66). TNF-alpha initiates the so-called Survivor Activating Factor Enhancement (SAFE) pathway which is characterized by activation of Janus kinase (JAK) and signal transducer and activation of transcription 3 (STAT-3) (66). To test the involvement of the RISK and SAFE pathways in melatonin-induced cardioprotection, hearts were subjected to ischaemia and freeze-clamped after 10 min of reperfusion (in the presence and absence of melatonin) and Western blotting applied (58; Lochner, unpublished data). Under these conditions the prosurvival kinases ERK1/2, PKB/Akt as well as STAT-3 were significantly phosphorylated (thus activated), with concomitant inactivation of the pro-apoptotic kinase, p38 MAPK.

Using a different experimental protocol (hearts were pretreated with picomolar quantities of melatonin, followed by washout before freeze-clamping), Lamont and coworkers (42) showed increased STAT-3 activation *before* the onset of sustained ischaemia to be associated with a reduction in infarct size during reperfusion. Involvement of TNF-alpha and STAT-3 in melatonin-induced cardioprotection was further demonstrated by the fact that hearts from TNF receptor 2 knockout and cardiac STAT-3-deficient mice could not be protected against I/R injury by melatonin (42). This observation lends a new perspective to the ability of melatonin to protect the heart, suggesting that activation of the SAFE pathway *before* the onset of ischaemia may also play a role in subsequent cardioprotection. Whether this holds true for PKB/Akt and ERK1/2 activation before ischaemia is not known. It is, however, known that administration of melatonin during reperfusion only can very effectively induce protection, suggesting that activation of these pathways before ischaemia is perhaps not a prerequisite (35).

The significance of ERK1/2 and STAT-3 activation either before or after ischaemia in melatonin-induced cardioprotection was confirmed by the fact that PD98059, an ERK1/2 inhibitor and AG490, a STAT-3 inhibitor, abolished protection (42, Figure 3).

6.2. Melatonin and mitochondria

6.2.1. Mitochondrial function

Mitochondria are considered the main intracellular source of ROS production and paradoxically, the major target of a free radical attack. During normal physiological conditions, ROS are generated at very low levels, but can increase dramatically in pathophysiological conditions such as myocardial I/R (67), causing a loss in the phospholipid cardiolipin, which in turn, is responsible for the loss of activity of the respiratory chain complexes I, III and IV and mitochondrial dysfunction (68,69).

Petrosillo and coworkers (70) demonstrated convincingly that, at pharmacological concentrations (50 microM), melatonin effectively protects against I/R

damage: it caused a reduction in lipid peroxidation and counteracted the reduction in State 3 respiration and the respiratory control ratio in rat heart mitochondria, isolated after exposure of the heart to I/R. These workers showed that the protection afforded by melatonin against mitochondrial dysfunction was associated with improvement in functional recovery during reperfusion.

6.2.2. Mitochondrial permeability transition pore

Cardioprotection associated with activation of the RISK and SAFE pathways at the time of reperfusion was demonstrated to be due to inhibition of the mitochondrial permeability transition pore (66,71).

Multiple studies suggest a role for the MPTP in apoptosis or necrosis with release of cytochrome C, as well as other proteins, from the mitochondria, playing a central role. Petrosillo and coworkers (72) recently reported that exogenous oxidized cardiolipin added to mitochondria, causes opening of the MPTP and cytochrome C release. A further study showed that oxidation of intramitochondrial cardiolipin molecules causes MPTP induction, which can be inhibited by melatonin (73).

These findings were confirmed by the observation made by Petrosillo and coworkers that melatonin protects the heart against I/R injury by inhibiting MPTP opening (74). It was found that mitochondria isolated from melatonin-perfused hearts were less sensitive than mitochondria from untreated reperfused hearts to Ca^{2+}-induced MPTP opening, as assessed by the calcium retention capacity of the mitochondria.

7. MYOCARDIAL SUBSTRATE METABOLISM

As far as we know, no information is available about the direct effects of melatonin on myocardial substrate metabolism in normoxia or during ischaemia/reperfusion.

The studies referred to above (35,42,58) used the isolated perfused heart as model with glucose as the only substrate present. It is well-established that glucose is an important source of energy during ischaemia, due to its ability to generate ATP in the absence of oxygen and the increased glycolytic ATP production may be sufficient to maintain ionic homeostasis in a model of regional ischaemia. Although the rate of glucose oxidation is usually depressed during reperfusion (for review see ref 75), it remains a major source of energy production and any stimulation of glucose uptake should be beneficial for recovery of the heart during reperfusion.

Whether the melatonin-induced increases in the activation of the survival kinases and STAT-3 during reperfusion are associated with changes in substrate metabolism, is not known. PKB/Akt (76) as well as ERK (77-79) activation may stimulate glucose uptake (79) while the JAK-STAT pathway does not seem to be involved in this process (80). However, as stated above, the effects of melatonin on myocardial substrate metabolism during reperfusion remains to be determined.

Despite the lack of information regarding the effect of melatonin on myocardial glucose metabolism, data obtained from experimental animals *in vivo* and *in vitro* suggest that the hormone contributes to the regulation of whole body glucose homeostasis. This is confirmed by the fact that pinealectomy induces insulin resistance and glucose intolerance (81,82). A recent study by Muhlbauer *et al* (83), using melatonin receptor knock out mice, indicated an active role of these receptors in the regulation of blood glucose levels. It has been reported several years ago that melatonin decreases insulin secretion *in vitro* (84) and *in vivo* (85), mediated by the MT_1 receptor. Melatonin effects on the pancreatic beta-cells are mediated by 3 intracellular pathways, viz. the Gi-alpha-protein dependent cAMP pathway, the cGMP pathway and by modulation of pathways activated by the muscarinic-acetylcholine receptor (86).

An important observation recently made was the fact that removal of the melatonin receptor type 1 (MT_1) significantly impairs the ability of mice to metabolize glucose and probably induces insulin resistance in these animals (87). Convincing evidence has been presented that melatonin stimulates the rate of glucose uptake by C_2C_{12} mouse myotube cells, via an IRS-1/PI-3K pathway which appears to be receptor mediated (88), with involvement of PKC, but not AMPK, activation (88,89). Despite the lack of experimental evidence on the effects of melatonin on myocardial glucose uptake, indirect evidence suggests a role for the hormone in this regard: it has been shown that melatonin treatment restores GLUT4 gene expression and glucose uptake in cardiomyocytes which were inhibited by administration of 3,5,3'-triiodo-L-thyronine (20). In addition, melatonin completely restores the level of MEF2, a regulator of GLUT4 transcription.

In contrast to the above, Sartori and coworkers (90) could not demonstrate an effect of melatonin on insulin-stimulated Akt phosphorylation or glucose uptake in skeletal muscle of normal mice *in vivo*. However, in a model of diet-induced insulin resistance and vascular dysfunction, melatonin markedly improved glucose homeostasis and restored endothelial vascular insulin signaling and responsiveness (84). These observations support the notion previously suggested that melatonin often exerts its effects in pathophysiological conditions, while having little effect in normal conditions (16).

In summary, it appears very likely that melatonin may directly affect myocardial glucose metabolism. Since myocardial substrate uptake is regulated by its circulating levels (91), the effects of melatonin on glucose homeostasis (83), may indirectly affect glucose uptake. In addition, the heart utilizes, amongst others, fatty acids as substrates, which can profoundly affect the outcome of I/R. As far as we know, the effect of melatonin on myocardial fatty acid metabolism has not been studied, neither in normoxia nor I/R.

8. MELATONIN AND INTRACELLULAR CALCIUM HANDLING

Melatonin has been suggested to modulate intracellular Ca^{2+} via activation of its G-protein coupled membrane receptors or through a direct, but weak,

interaction with calmodulin (92). Using confocal microscopy and the fluorophore fluo3, it has been shown that melatonin reduced intracellular calcium in cardiomyotyes exposed to chemical hypoxia and caused a reversal of hypoxia-induced morphological changes (93).

It is known that myocardial infarction is associated with a reduction in the activities of membrane-bound Na^+K^+ATPase and Mg^{2+}ATPase and an increase in Ca^{2+}ATPase activity (15), all of which were reversed by melatonin pretreatment. In addition, the cardioprotective effects of melatonin were associated with reduced TNF-alpha levels and myeloperoxidase (MPO) activity (94) which could be very important in view of the harmful effects of TNF-alpha on altering calcium homeostasis, excitation-contraction coupling, NO metabolism and signaling (93). This, however, is in contrast with the suggestion that the TNF-alpha receptor is involved in melatonin-induced cardioprotection (42) and should be further investigated.

8.1. Melatonin and the sarcoplasmic reticulum

It has recently been shown that melatonin ameliorates changes in calcium homeostasis which were observed in rat hearts exposed to chronic hypoxia (96). Cardiomyocytes isolated from such hearts exhibited increases in resting calcium levels and I/R-induced calcium overload, impairment of the function of SR calcium handling proteins as well as attenuation of SERCA protein expression. Cardiomyocytes isolated from hearts of melatonin-treated hypoxic rats, showed that melatonin treatment preserved the SR-calcium content and prevented downregulation of SERCA expression. Thus melatonin treatment significantly mitigated the calcium handling in hypoxic hearts by preserving SERCA expression. However, the involvement of the anti-oxidant effects of melatonin in SERCA regulation remains to be established.

In contrast to the above, long-term administration of melatonin to rats after coronary artery ligation, may have different effects on Ca^{2+}homeostasis: exogenous melatonin administration diminished the mRNA expression of the dihydropyridine receptor (DHPR), the ryanodine receptor (RyR) and SERCA2 proteins, while a 1.9 fold increase in the level of MT_2 proteins was observed (97). Unfortunately these observations were not correlated with SERCA function and intracellular calcium homeostasis. It was suggested that these changes contributed to the beneficial actions of melatonin postinfarction. The fact that melatonin elicited different responses in different pathophysiological conditions needs to be further investigated.

8.2. Melatonin and ANP

The above study also showed that melatonin administered postinfarction, increased the concentration of left ventricular ANP to over 5-fold. It is well-established that myocardial infarction itself leads to a profound increase in the release of ANP (for a review see ref 98) which has vasodilating, diuretic and natriuretic effects (98). It is possible that the increased LV ANP levels after myocardial infarction adds one more way by which melatonin can protect the heart against I/R injury. Clearly, this possibility needs to be further investigated.

9. CONCLUSIONS

It can be stated that the pineal gland hormone, melatonin, is indeed an effective cardioprotectant against myocardial ischaemia/reperfusion damage. This may be due to its free radical scavenging and anti-oxidant properties, but there is also convincing evidence for its receptor-mediated actions. In this regard the powerful anti-adrenergic actions of melatonin may be of importance in protecting the heart against damage.

Although very little is known about the effects of melatonin on myocardial substrate metabolism in both normoxia and in I/R, observations made in pinealectomized and melatonin receptor knockout animals suggest that the hormone may have a profound effect on glucose metabolism. This may occur indirectly in view of the effects of circulating substrate levels on the uptake thereof by the heart.

Finally, recent observations indicated participation of the RISK and SAFE pathways in the cardioprotective actions of melatonin. Whether these two pathways converge on the mitochondrial permeability transition pore remains to be established, but experimental evidence suggests that melatonin, similar to many other cardioprotective interventions, exerts its effects via this, as yet uncharacterized, protein channel.

10. ACKNOWLEDGMENT

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Abbreviations: NO: nitric oxide; PKC: protein kinase C; PKB: protein kinase B; ERK1/2: extracellular signal regulated kinase; RISK: Reperfusion Injury Salvage Kinase; STAT-3: signal activator and transducer 3; SAFE: Survivor Activating Factor enhancement; MPTP: mitochondrial permeability transition pore; I/R ischaemia/reperfusion; MPO: myeloperoxidase; MDA: malondialdehyde; PDE: phosphodiesterase; cGMP: cyclic guanyl monophosphate; cAMP: cyclic adenosine monophosphate; PKA: protein kinase A; TNF-alpha: tumor necrosis factor alpha; JAK: Janus kinase; p38MAPK: mitogen activated protein kinase; IRS-1: insulin receptor substrate-1; PI-3K: phosphatidylinositol-3-kinase; AMPK: adenosine monophosphate kinase; GLUT4: glucose transporter 4; MEF2: myocyte enhancing factor 2; SERCA: Sarco(Endo)plasmatic Reticulum calcium ATPase; DHPR:

dihydropyridine receptor; RyR: ryanodine receptor; ANP: atrial natriuretic peptide

Key Words: Myocardial Ischaemia/Reperfusion, Myocardial Substrate Metabolism, Glucose Metabolism, Melatonin Receptors, RISK pathway, SAFE pathway, Review

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