Review

Melatonin as a Potential Antiarrhythmic Drug: Myocardial Ischemia and Beyond

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Melatonin as a Potential Antiarrhythmic Drug: Myocardial Ischemia and beyond

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Abstract: Life-threatening cardiac arrhythmias such as ventricular tachycardia and/or fibrillation often complicate myocardial ischemia and constitute a cause for sudden cardiac death. Development of strategies for the prevention of such arrhythmias present an important research problem. The use of classical antiarrhythmic drugs is related to significant risk due to their aggressive action and probable proarrhythmic side-effects. Such risks are generally affordable in urgent situations, but unacceptable for preventive use in large patient populations. It poses the necessity to develop a safe and effective medication to be applied for arrhythmia prophylaxis and treatment. Melatonin has been proved to be safe for use in humans even in relatively high doses. It has been reported to confer versatile cardioprotective effects, which include antiarrhythmic action. Traditionally, the antiarrhythmic effect is considered as a manifestation of the general protective action of melatonin, based largely on its antioxidative properties. In this review, we strive to provide evidence of specific electrophysiological mechanisms brought about by melatonin application. Here, we discuss the pathways by which melatonin targets ion channels and causes changes in the cellular and tissue properties relevant to arrhythmogenesis.The obtained data suggest that melatonin can modify a number of electrophysiological characteristics of a cardiomyocyte and myocardium. However, the changes in conduction velocity appear to be the major effect functionally relevant for arrhythmogenesis. The most probable mechanisms of the melatonin-related conduction enhancement are discussed. These mechanisms differ in acute and chronic applications of melatonin and related to the increase of inward rectifier potassium current maintaining the resting membrane potential and the upregulation of sodium channel protein expression, respectively.The novel melatonin-related mechanism regulating myocardial conduction velocity might be considered for potential use in different contexts that include arrhythmia risks from myocardial ischemia to inherited channelopathies. The functional relevance of this effect warrants its further exploration concerning cellular mechanisms, efficiency and limitations.

Keywords: melatonin; arrhythmias; ischemia; reperfusion; conduction velocity; dispersion of repolarization; ionic currents; oxidative stress

1. Introduction

Melatonin is an indole hormone, which is synthesized from tryptophan in the pineal gland and some extrapineal tissues. It binds membrane G-protein coupled receptors (MT1 and MT2), interacts with a number of intracellular molecular targets, which can mediate its signaling effects, and scavenges reactive oxygen species (ROS) thereby conferring antioxidative defense. These multifaceted effects are categorized as receptor-dependent and receptor-independent actions, respectively. Melatonin is a widely spread molecule in living organisms, both animals and plants [1]. For the first time, it has been identified as an active compound of the pineal gland extract responsible

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for discoloration of the skin in frogs [2]. This effect was found to be regulated by the intensity of ambient light and therefore has been considered as an adaptation mechanism to the changes in illuminance in lower vertebrates (for review, see [3]). Excretion of melatonin in humans was observed to demonstrate circadian oscillations [4], which led to a suggestion that its production follows the same rhythm. Indeed, in the pineal gland, it is enhanced at darkness and suppressed at light by the changes in the activity of the rate-limiting enzyme serotonin N-acetyltransferase (SNAT) (also referred to as arylalkylamine N-acetyltransferase (AANAT) in vertebrates) [5]. Currently, the role of melatonin as a master entraining agent in day/night and annual rhythmicity is generally accepted. As such, it regulates circadian cycle of behavioral activity, sleep/wakefulness and maturation of seasonally breeding animals [6].

Apart from its "classical" rhythm-control effects, melatonin can serve other functions across the organism. Due to its physicochemical properties, melatonin easily crosses biological barriers. Moreover, melatonin production in peripheral tissues can exceed its pineal production severalfold, and as a result, melatonin is widely distributed in the body, with its content in different organs varying in a wide range [7]. Melatonin receptors are also found ubiquitously [8], and membranes are permeable for melatonin, which favors its transport into different cellular compartments including mitochondria [9, 10]. These properties form the basis for versatile melatonin functions in the body, many of which can be described as protective, since melatonin scavenges dangerous oxygen and nitrogen species, fosters antioxidant systems, controls acute and chronic inflammation, and confers oncostatic effects.

Among these multifaceted effects, melatonin has been reported to confer cardioprotection. These effects were observed in different pathological conditions, like acute ischemia, ischemia-reperfusion, arterial hypertension, exposure to cardiotoxic substances, heart failure to name just a few. Melatonin-related cardioprotection includes antiarrhythmic action. Theoretically, it might be ascribed both to general protective effects, for example, ROS scavenging and/or to direct modification of electrophysiological properties, an effect, which implies the presence of certain signaling pathways from melatonin to specific electrophysiological targets, like ion channels. Probably, the critical point in understanding the antiarrhythmic effects of melatonin is discerning between these two possible mechanisms, which do not exclude each other. This review aims to discuss evidence on the direct effects of melatonin on electrophysiological properties of cardiomyocytes which can account for its antiarrhythmic activity (mostly concerned with ischemia) and might be possibly used for the development of novel antiarrhythmic drugs and arrhythmia management strategies. Mostly, we will focus on ventricular tachyarrhythmias, specifically ventricular tachycardia and/or ventricular fibrillation (VT/VF), which complicate myocardial ischemia. However, other aspects of potential melatonin applications will be also shortly addressed.

2. Ischemic Arrhythmogenesis

2.1. Reentry as a Mechanism of Life-Threatening Arrhythmias

Normally, the heart is excited by the activation wave, which spreads from the sinoatrial node to the atria, atrioventricular node, ventricular conduction system and finally to the contractile ventricular myocardium until it is extinguished after the entire muscle is activated. A period of excitation is followed by an electrically silent period until the next sinoatrial node discharge. This activation pattern ensures periodical contractions and relaxations underlying normal pump function of the heart. Life-threatening arrhythmias, i.e. VT/VF, result from the so-called reentry mechanism, which means that the activation wave continuously circulates in the ventricular myocardium rendering periodical pumping impossible. This phenomenon presents a main cause for the sudden cardiac death, which usually arises as a complication of cardiovascular diseases and constitutes a great public problem [11-13].

In general, the mechanism of reentry is considered as the interaction between an arrhythmogenic substrate and a trigger. The concept of the substrate includes three major properties of myocardium, (1) the presence of an inexcitable myocardial region (core), around which the activation wave travels;

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(2) unidirectional conduction block, which prevents collision of activation waves; (3) excitable gap in front of the activation wave. The inexcitable region can be presented by either an anatomical obstacle or refractory tissue. The unidirectional conduction block usually develops as a result of dispersion of repolarization (DOR), (strictly speaking, dispersion of refractoriness). The spatial excitable gap is the difference between the anatomical length of the activation trajectory and the excitation wavelength, which is the product of the conduction velocity and action potential duration (APD) (strictly speaking, refractory period). In other words, the excitable gap is a part of the circular activation path ahead of activation wavefront, which is not yet excited. The trigger of reentry is usually presented by a premature ventricular beat (PVB), which falls into the so-called vulnerable period, determined by DOR. It means that PVB could trigger reentry, if it develops within the time interval when a part of myocardium is already recovered from refractoriness, whereas the other part is still refractory.

2.2. Major Proarrhythmic Changes During Ischemia and Targets for Antiarrhythmic Treatment

Myocardial ischemia is a typical arrhythmogenic condition since it provides major prerequisites for reentrant arrhythmogenesis [14-16]. Obviously, the ischemic, necrotic or scar tissue presents an obstacle to be passed around by the activation wave. The distribution of refractoriness becomes highly heterogeneous in the ischemic conditions due to the opposite changes in the ischemic and border areas. Depolarization of the resting membrane potential (RMP) decreases sodium channel availability and prolongs refractory period even beyond the termination of the action potential (postrepolarization refractoriness). Action potential duration (APD) predominantly shortens in ischemia; however, transient APD prolongation is observed during first minutes after ischemia induction [17, 18]. These changes in APD entail corresponding changes in refractory period duration. As a result, normal, prolonged and shortened duration of repolarization and refractoriness could be observed in the locally ischemic myocardium, which provides conditions for the unilateral conduction blocks. Also, ischemia causes the increase in the excitable gap in two ways, namely slowing conduction due to the inactivation of sodium channels [19, 20] and suppression of connexin conductivity [21, 22] and APD shortening mainly due to the opening of K(ATP) channels and activation of the IK(ATP) current, which is suppressed in the normal conditions by a high enough level of intracellular ATP [23, 24]. Ectopic activity serving as reentry triggering can be evoked by several mechanisms, including abnormal automaticity [25] , early [26] or delayed [27] afterdepolarizations , and the so-called phase 2 reentry (where electrotonic depolarizing current flows between normal and affected myocardium) [28, 29]. For the purpose of this particular review, it is noteworthy that RMP depolarization [25] and sympathetic activation [30, 31] during ischemia can serve as primary factors, which precipitate the development of reentrant triggers.

A border zone between the normal and ischemic myocardium presents a distinct set of proarrhythmic properties. Usually, it demonstrates abnormal and often heterogeneous repolarization with relatively preserved conduction properties [32-35]. The border zone contributes much to the DOR as it could have both prolonged and shortened APD and refractoriness. Moreover, the duration of repolarization in the border zone could have arrhythmogenic consequences, which are independent of DOR [36, 37], since prolonged APD in the border zone predispose to early afterdepolarizations, which can trigger the reentry. Since the border zone is at least partially supplied with blood even during the episode of ischemia, its properties can be modified by therapeutic agents delivered to tissues by blood flow.

Before a detailed discussion of potential antiarrhythmic effects of melatonin, some distinctions of the ischemia (ischemia-reperfusion) setting should be identified. It is important that under coronary occlusion the agents that are transported by blood flow cannot access the core ischemic area. It means that melatonin, if acutely infused before opening the coronary artery can act only on the nonischemic myocardium or the border zone, which should have at least partial blood supply. This reservation does not necessarily mean that blood-borne agents are unable to confer antiarrhythmic effects in these conditions. As was discussed above, the arrhythmogenic substrate requires the presence of electrophysiological heterogeneity. This heterogeneity is formed due to the differences between the normal and affected myocardium and therefore can be changed by the modification of

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not only the affected region but also the normal myocardium. Moreover, the border zone, which is at least partly supplied with blood flow, can present a promising target for antiarrhythmic drugs.

On the other hand, melatonin administered during ischemia can access the affected region after the artery opening at reperfusion, which is widely used as a provocative test for arrhythmias including the studies of melatonin. However, the melatonin ability to influence the reperfusion outcomes depends on timing of reperfusion arrhythmias evaluation, which is often short and limited to several minutes, timing of melatonin administration in the period of ischemia and the rate of melatonin distribution and/or elimination. The half-life time of melatonin is estimated to be several minutes in the case of intravenous injection and less than 1 hour in oral administration [38]. It is noteworthy that unlike experimental models, which strive to exploit standard protocols, timing of ischemia, reperfusion and treatment may vary considerably in clinical settings.

Therefore, in acute application during the ischemic episode the potential of melatonin for modification of the affected region is limited; on the other hand, melatonin can influence the normal and border zones. The situation is different when an experimental animal or a patient is pretreated with melatonin before the ischemic episode. In such a case, melatonin is able to modify the entire myocardium. However, the effects are expected to depend on the duration of pretreatment. Anyway, the issues of the timing should be taken into account when the results of melatonin application are interpreted.

2.3. ROS-Dependent Arrhythmogenesis

Ischemia and particularly ischemia-reperfusion episodes are strongly associated with the development of oxidative stress, which brings about a distinct set of arrhythmogenic mechanisms. The obvious consequence of the oxidative stress is myocardial damage, which can reinforce almost all the ischemia-related proarrhythmic changes listed above. However, ROS generation may induce specific electrophysiological changes, which may result in arrhythmias via the influence on potassium and sodium currents, calcium homeostasis and impulse conduction [39].

A ”metabolic sink” is one of these ROS-related arrhythmogenic mechanisms [40, 41]. The “metabolic sink” theory suggests that during ischemia-reperfusion a superoxide anion is formed and flows through the internal membrane anion channels (IMAC), which leads to a collapse of the mitochondrial ΔΨm. As a result, ATP synthesis significantly decreases and the sarcolemmal IK(ATP) current is activated. Due to the abundance of the K(ATP) channels in cardiomyocytes, the increase in this current results in (i) the significant shortening of action potential duration leading to the shortening of the excitation wavelength and likely the increase in the DOR and (ii) the lock of the membrane potential at the level near the potassium reversal potential, reducing cellular excitability and providing the current sink for the spreading activation wavefront [42, 43].

Another arrhythmogenic mechanism could be related to the modification of sodium current. The inhibition of INa by ROS generated by mitochondria [44, 45] potentially leads to conduction slowing. Moreover, oxidative stress and ROS generation can facilitate an arrhythmogenic late sodium current (INa,L), a so-called window current, caused by the relative shift of the sodium current steady-state inactivation curve to more positive potentials [46]. Recently, it was shown that this effect is likely more relevant not for the typical cardiac Nav1.5 channel, but for minor tetrodotoxin-sensitive Nav1.2 and Nav1.3 channels [47]. INa,L leads to the lengthening of action potential duration and predisposes to early afterdepolarizations. It was demonstrated that the ROS-induced facilitation of INa,L was mediated by the CaMKII-dependent pathways [48, 49]. However, the sole activation of INa,L by ROS-CaMKII-dependent cascade can account for APD prolongation but not necessarily the generation of early afterdepolarization, the latter requires simultaneous activation of INa,L, calcium and probably sodium-calcium exchange currents [50, 51]. In addition to the facilitation of INa,L APD prolongation and early afterdepolarizations could be accounted for by the ROS-dependent inhibition of potassium currents [52-55]. On the other hand, it is noteworthy that although APD prolongation is possible during ischemia, especially at its earliest stages, the most typical and pronounced change in APD in ischemia is shortening. It implies that the discussed above mechanisms are relevant to reperfusion, rather than ischemia itself.

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Several lines of evidence point at calcium homeostasis disorder caused by ROS as an arrhythmogenic mechanism in ischemia-reperfusion. ROS, and specifically H2O2, have been shown to increase cardiac sodium-calcium exchanger-dependent Ca^{2+} influx [56], which could cause the calcium cellular overload having pleiotropic arrhythmogenic consequences, e.g., afterdepolarizations serving as triggers for the malignant ventricular arrhythmias. The deterioration of the sarcoplasmic reticulum calcium-regulating proteins could be caused by ROS [57, 58] and, on the other hand, the correction of redox cellular state improves the calcium handling [59].

Taken together, these data suggest that oxidative stress predisposes to reentrant and nonreentrant arrhythmias facilitating the development of unidirectional block, activation slowing, formation of inexcitable regions and triggered activity.

3. Antioxidative Properties of Melatonin

ROS are formed during normal cellular processes and should be neutralized to avoid their detrimental effects. In ischemia and particularly reperfusion, ROS generation is greatly increased, and the complexes I and III of the electron transport chain (ETC) in mitochondria are considered to be the major source of ROS (for review see: [60-62]). During ischemia, a relatively small amount of ROS is produced by the ETC. This effect possibly stems from mitoK(ATP) channel opening, which leads to the partial substitution of protons with potassium ions. The resulting alkalinization of mitochondrial matrix retards the transport of electrons from complex I and shunts them to the molecular oxygen [63]. The generated superoxide anion and downstream ROS cascade damage the ETC [64] leading to a much more vigorous generation of superoxide anion during reperfusion [65]. Together with calcium overload, ROS generated in mitochondria can directly open the mitochondrial permeability transition pore inducing cell death by necrosis and apoptosis and indirectly leading to arrhythmogenic mechanisms summarized above.

ROS are highly reactive and unstable compounds, moreover, some of them are charged and thus could not readily travel across membranes. These properties underlie a concept of the so-called compartmentalization of the oxidative stress, i.e. the major detrimental and/or signaling effects of ROS tend to occur within the compartment where they were generated [39, 66]. It means that to provide benefit, antioxidant agents should not simply load an organ or cell at risk, but have to be delivered to the very place of ROS formation, in the context of ischemia-reperfusion to mitochondria.

Melatonin serves several physiological functions and antioxidant effects conferred by melatonin are considered as its first function in biological evolution [9]. Melatonin is recognized as a potent, versatile and regenerating antioxidant [67]. Due to its amphiphilic properties, melatonin could readily enter the cell and mitochondria. Due to these properties, the role of melatonin as an antioxidant can be even more important. Melatonin has been reported to confer organ protection due to its versatile antioxidative effects [68, 69] mediated by both direct ROS-scavenging [70] and stimulation of the antioxidative enzymatic activity [71-73]. These regulatory antioxidative effects are mediated by the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway [74-76], a major transcription factor responsible for the activation of multiple protective signaling cascades including antioxidative cascades [77].

Due to its amphiphilic properties, melatonin could readily enter the cell and specifically mitochondria and reduce mitochondrial ROS generation, restore the mitochondrial glutathione homeostasis (in contrast to other potent antioxidants like vitamins C and E) [78, 79] and maintains the activity of ETC complexes I, III and IV in oxidative stress [80, 81]. The latter effect favors the flow of electrons along the ETC thereby avoiding their leak to molecular oxygen and generation of superoxide anion, a unique action of the indole. Melatonin confers a mild decrease in oxygen consumption small enough to not cause ATP depletion, but sufficient for the attenuation of ROS production by mitochondria [82, 80]. It also diminishes the calcium and ROS-induced permeability transition pore opening [83] preventing ATP depletion and apoptotic pathways triggering.

Among other organs, melatonin targets the heart and confers antioxidative effects in myocardium [84]. Melatonin has been reported to protect against injuries of different origins associated with oxidative stress. Among others, the conditions where melatonin demonstrated its

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antioxidative effects include obesity [85, 86], sepsis [87-91], doxorubicin toxicity [92-94, 74], pulmonary arterial hypertension [95]. Melatonin's specific attenuation of ischemia-reperfusion-related oxidative stress has been widely studied [84, 96-100]. Similarly to other organs and tissues, melatonin influences redox state of cardiomyocytes directly as a ROS scavenger and indirectly as an activator of the antioxidative signaling pathways resulting in the upregulation of the antioxidative enzymes. This indirect melatonin-related mitigation of oxidative stress and apoptosis has been demonstrated to be mediated by MT2 but not MT1-dependent pathway [101, 102]. Alleviation of mitochondrial function is thought to be the most probable underlying mechanism of antiischemic cardioprotection.

Taken together, the obtained thus far findings suggest that melatonin confers significant antioxidative effects. Mitochondria can be considered as the major end-target for melatonin as an antioxidant. These actions are expected to be converted into cell- and organ-protection, which should be based on the alleviation of oxidative stress. It is noteworthy that melatonin counteracts oxidative stress directly and indirectly via regulation of the expression of antioxidative enzymes. These two pathways of protection are not easily discerned from each other, and the latter one requires preserved signaling cascades for its functioning.

4. Melatonin as a Signaling Molecule

4.1. G Protein-Coupled Plasma Membrane MT1 and MT2 Receptors

There are several subtypes of membrane melatonin receptors, the two of which are best described, namely MT1 and MT2. A melatonin-related receptor (GPR50) though being a member of the melatonin receptor subfamily does not bind melatonin (or any other known ligand) and thereby presents as an orphan receptor. The major effects of both melatonin receptor types are accounted for by their coupling to Gi protein resulting in the decrease of cAMP production by adenylyl cyclase, which is usually estimated as a decrease in the forskolin-induced cAMP production response [103]. MT1 receptor can be also coupled to Gq proteins, although this interaction is significantly less common [103], probably due to lower affinity of the bond and cell-specificity [104, 103]. Also, melatonin has been shown to activate PI3K/Akt and ERK1/2 pathways via MT1 and/or MT2 receptors [105, 106] and therefore positively regulate transcription of genes controlled by these pathways [107].

MT1 and MT2 receptors although having a lot of common effects, due to the fact that they share Gi-dependent transduction cascade, do possess functional distinctions. It has been demonstrated that MT1, but not MT2, receptor can couple to Gs-protein as well [108]. On the other hand, MT2, but not MT1, receptor also inhibits the formation cGMP by soluble guanylyl cyclase [109]. These minor pathways are expressed differently in different tissues. This diversity might be considered as a manifestation of system-biased signaling in the melatoninergic system, which provides necessary flexibility in regulating physiological functions.

Melatonin receptors can be constitutively active, and their antagonists, like luzindole, can act as an inverse agonists [110]. Such effects were demonstrated in preparations of rat arteries isolated during the light phase of circadian cycle, i.e. with the minimal systemic level of melatonin prior to the isolation [111]. The fact that an antagonist of the receptor evokes a response (which is opposite to that of an agonist) when receptor-agonist binding is precluded confirms the presence of the constitutive activity. The inverse agonistic effects of receptor antagonists were also observed in human MT1 and/or MT2 receptors expressed in CHO [112, 113], HEK293 [114] and Neuro2a [113] cell lines. The molecular basis for this phenomenon was established to be a tight association of the receptor with Gi protein, which was independent of the presence of agonists [114]. However, the functional role of the constitutive activity remains still not clear.

Melatonin receptors can undergo oligomerization forming homo- and heteromers (MT1/MT1, MT1/GPR50, MT1/MT2, MT2/MT2), which represent agonist binding-independent preformed GPCR–effector macromolecular membrane assemblies [103]. The propensity of MT1/MT1 and MT1/MT2 dimerization is severalfold higher than that for MT2/MT2 [115]. MT1/MT1 homodimers signal via Gi-coupling, i.e. in a similar way as the MT1 monomer, whereas the MT1/MT2 heterodimer

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activates Gq – phospholipase C – protein kinase C-dependent transduction chain [115, 116]. GPR50 orphan receptor oligomerization with MT1 receptor abolishes agonist binding by the MT1 and therefore inactivates the MT1-dependent signalling cascade [117]. MT2 receptor can also form heterodimers with serotoninergic 5-HT2C receptors, where melatonin transactivates Gq-dependent pathways normally related to the 5-HT2C receptors [118]. Luzindole and 4P-PDOT, which are competitive antagonists of MT1 and MT2 receptors in the absence of the 5-HT2C receptor, behave as agonists of the cAMP pathway and full or partial agonists, respectively, of the IP pathway in cells expressing the MT2/5-HT2C heteromer [110].

Both MT1 and MT2 receptor types are present in the myocardium [119], though the level of their expression in the heart is relatively low as compared to other tissues [120, 121]. However, the effects of their activation or inhibition in the myocardium have been reported [101, 122, 102, 123, 124]. MT2 has been consistently reported as a more abundant receptor subtype in the myocardium.

4.2. Other Signaling Pathways

Since melatonin can enter the nucleus, the existence of nuclear melatonin receptors has been suggested. However, the identification of such receptors appeared challenging. Retinoic acid-related orphan receptors (ROR) were considered as promising candidates, but findings concerning their role are controversial [125-128]. There are reasons to believe that ROR activation by melatonin is indirect. Recently, vitamin D receptors (VDR) have been reported to bind melatonin [129] and thereby might function as the nuclear melatonin target, which warrants further exploration of the VDR role in melatonin signaling.

Melatonin can also bind some cytoplasmic proteins. Quinone reductase 2 is also referred to as cytoplasmic melatonin receptor MT3. The downstream effects of MT3 activation are unclear, but they are probably related to ROS scavenging by the enzyme [98], which converges with the antioxidative action of melatonin itself. Moreover, melatonin can modify the activity of calmodulin-dependent kinase II (CaMKII) [130]. Melatonin can directly interact with calmodulin [131] mostly decreasing the activity of the latter. However, calmodulin and CaMKII may serve as a part of MT1/MT2 receptor-dependent signaling pathway [132]. Also, melatonin can affect calmodulin distribution in the cellular compartments [133]. These complex interactions account for both inhibition (mostly short-term) and activation (mostly long-term) of the CaM-dependent pathways by melatonin.

Calcium ions are involved in several signaling pathways, including but not limited to Ca2+-CaM/CaMKII and protein kinase C pathways. Directly or indirectly, melatonin can modify intracellular Ca2+ concentration and thereby influence intracellular enzymatic machinery. Calcium permeability is a common characteristic of most members of the transient receptor potential (TRP) channel family members. TRP channel subtypes V1, V2, C3-C7, M2, M4, M7, PP2 were found in the myocardium (for review, see [134]). Melatonin has been demonstrated to downregulate TRPC6 [135] and TRPV1 [136, 137] channels in noncardiac tissues, and similar effects in cardiomyocytes may be expected. It is noteworthy that many TRP channels can be activated (upregulated) by ROS, [138] which in turn are scavenged by melatonin. Moreover, melatonin might directly or indirectly participate in store-operated calcium entry operating via influence on TRP channels.[135, 139] Finally, melatonin also downregulates voltage-gated calcium channels in cardiac and other tissues [140, 141].

Taken together, the available data suggests that melatoninergic signaling via G protein-coupled plasma membrane MT1 and MT2 receptors is best described. Other signaling cascades are much less elucidated; they also may converge with GPCR-related pathways and antioxidative processes, and therefore their functional role, though possible, remains largely unclear thus far.

5. Arrhythmogenesis-Related Effects of Melatonin

A number of studies have demonstrated that melatonin reduces the incidence, duration and severity of ventricular arrhythmias including life-threatening tachyarrhythmias, such as VT/VF. Probably, the first report on the antiarrhythmic effect of melatonin appeared as early as in 1979 [142], four years later than the circadian rhythm of melatonin production had been discovered [4]. Specifically, it was shown that melatonin prevents arrhythmias in the ischemic (ischemia-

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reperfusion) conditions [143-149]. However, the electrophysiological mechanism of this antiarrhythmic activity remains incompletely understood. In principle, three major mechanisms can be suggested for the melatonin antiarrhythmic effects. (i) Melatonin can alleviate the ischemic (ischemia-reperfusion) injury and indirectly attenuate arrhythmogenesis. (ii) By counteracting the oxidative stress, either directly by scavenging ROS or indirectly by activating cellular antioxidative systems, melatonin can suppress ROS-related arrhythmogenic mechanisms such as metabolic sink mechanism etc (see above). (iii) Melatonin might modify cellular electrophysiological targets such as ion channels in a way that precludes arrhythmias. These hypothetical mechanisms do not exclude and could complement each other.

5.1. Indirect Melatonin-Related Antiarrhythmic Mechanisms are Possible But Still not Confirmed

Melatonin improves the conditions of the heart in the ischemia-reperfusion model (for review see: [150-152, 98]). It protects myocardium against ischemia-reperfusion damage and/or reduces the infarct size via modifying the redox status (see above) , preventing Ca^{2+}-overload [84], inhibiting of the permeability transition pore opening [153]. In nonischemic conditions, melatonin causes no or little changes in hemodynamics [142, 146, 154]. In ischemia-reperfusion, melatonin improves the functional recovery of systemic hemodynamics, decreasing contractility and increasing lusitropy indices [147, 154]. The latter should decrease myocardial oxygen demand and limit the ischemic damage. Whatever the exact cardioprotective mechanism, the limitation of the size of the affected region will shorten the anatomical length of the activation pathway around the inexcitable core, which reduces the excitable gap between the activation wavefront and the refractory tissue (activation “head and tail”) and therefore decreases the probability of the formation of sustained reentry. However, no associations were found between the VT/VF incidence and the area of the ischemic region [155]. Moreover, antiarrhythmic effects of melatonin are often evaluated in the ischemia-reperfusion models with short durations of ischemia and reperfusion. In such models, the occlusion site (and therefore the volume of nonperfused myocardium) is usually standard, necrosis is unlikely and consequently the melatonin-related reduction of the infarct size could hardly manifest due to time limitation.

Earlier studies [96, 156, 145, 146, 148] directly ascribed the antiarrhythmic effects of melatonin to its ability to alleviate the oxidative stress. Such an interpretation is reasonable in light of considerations summarized above. Firstly, since the oxidative stress contributes to myocardial injury, application of melatonin can reduce the damaged region [145] and therefore decrease the size of the inexcitable zone, which is the prerequisite of reentry. Secondly, since ROS can induce the proarrhythmic changes via the metabolic sink mechanism (see above), the melatonin treatment could modify the changes of the APD and DOR during ischemia. Indeed, in our previous study [155], we found that chronic melatonin treatment enhanced the superoxide dismutase activity in the rat ischemia-reperfusion model, and this effect was associated with the changes in repolarization duration. However, the same study has shown that this effect was not associated with the VT/VF incidence in this model. Furthermore, our studies in a porcine [157] and rat [123] models of myocardial ischemia with the acute application of melatonin after coronary occlusion demonstrated its antiarrhythmic action without the effect on oxidative stress parameters. It is also of note that in spite of vast promising results of experimental and preliminary clinical studies, interventional clinical trials utilizing antioxidant supplementation have not confirmed the idea that the correction of redox status would decrease the cardiovascular mortality, including that from coronary heart disease [158-160].

Collectively, the obtained data suggest that the influence of melatonin on arrhythmogenesis was not directly related to its well-known antioxidative properties. Similarly, although melatonin indeed confers cardioprotective effects in the ischemic (ischemia-reperfusion) conditions, this protection does not necessarily convert into the antiarrhythmic action.

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5.2. Melatonin Effects on Cellular Electrophysiological Targets

At least part of cardioprotective effects of melatonin is mediated by melatonin receptors [154, 123]. Specifically, the role of MT2 receptors in the melatonin-dependent prevention of ischemia-reperfusion arrhythmias has been demonstrated [161]. It implies that the activation of the melatoninergic signaling pathways leads to the modification of cellular targets responsible for the properties of the arrhythmogenic substrate and triggering, for example, impulse conduction velocity, DOR, APD and refractoriness, PVB generation. In several experimental models, it has been found that melatonin treatment is able to modify all these characteristics in one way or another. However, both in chronic [162, 155] and acute [163, 123, 157] settings it was demonstrated that the only effect, which was independently associated with the decrease in arrhythmia incidence conferred by melatonin was the enhancement of impulse conduction. To substantiate this notion, below we summarize the data concerning the electrophysiological effects of melatonin obtained thus far.

5.2.1. Conduction Velocity (Chronic Effects)

Conduction velocity can be directly measured by epicardial mapping under electrical pacing and also can be assessed by indirect indices, such as local activation times in myocardial electrograms and duration of the QRS complex in electrocardiogram. It is characterized by anisotropy, i.e. the difference between its longitudinal and transverse components. The anisotropy is accounted for by the predominantly polar distribution of gap junctions responsible for electrical coupling between the cardiomyocytes. Melatonin has been reported to influence several characterisctics of myocardium, which affect conduction velocity. These properties include the extent of fibrosis and the properties of connexins and sodium channels. Fibrosis develops as a response to myocardial injury and inflammation, increases extracellular resistance and decreases conduction velocity. The connexins, specifically Cx43 in the contractile myocardium, are the main constituent of the gap junction channels coupling adjacent cardiomyocytes. The state of the connexin channels determines the intracellular resistance, which significantly increases in ischemia resulting in conduction slowing. Sodium channels determine cardiomyocyte excitability and conduction velocity. In the cell, they usually colocalize with connexins. When the RMP depolarizes in ischemia the availability of sodium channels drops due to inactivation and conduction slows down drastically.

The effects of melatonin on the synthesis of collagen and development of connective tissue in the heart differ in vivo and in vitro. Myocardial fibrosis has been demonstrated in pinealectomized rats [164] and melatonin receptor knockout mice [165] implying the negative influence of melatonin. In mice with dilated cardiomyopathy the level of melatonin was associated with the markers of inflammation and fibrosis, which was ameliorated after melatonin treatment [166]. Melatonin treatment also attenuated myocardial fibrosis in catecholamine stressed rats [167] and in pulmonary arterial hypertension [95], pressure overload-induced cardiac hypertrophy [168]. On the other hand, melatonin has been reported to increase collagen content in human [169] and rat [170, 171] cultured cardiac fibroblasts and myofibroblasts but not in the myocardium. It means that the effects of melatonin on the connective tissue deposition depends on the model used, and the direct effects on cells could be significantly modified by systemic mechanisms, for example the antiinflammatory effects of melatonin. It is also important that any effects on the development of fibrosis are expected to occur over a longer time scale as compared to that for acute coronary syndrome and might be irrelevant to the acute ischemic conditions.

Melatonin has been reported to improve the function of Cx43. Long-term melatonin treatment (at least several days) usually enhances total expression of Cx43 [172, 173, 167]. It also prevents Cx43 lateralization [172, 173, 167], which should maintain anisotropy of conduction. Finally, melatonin promotes Cx43 phosphorylation [172, 173], which may have different functional consequences depending on the kinases involved and the sites of phosphorylation. In our study [174], the total expression of Cx43 did not change under a week-long melatonin treatment, which means that the effect on Cx43 depends on the model used. Thus, the influence of melatonin on Cx43 appears to be complex and functional tests are needed to clarify the relationship between these effects and conduction velocities. Unfortunately, to the best of our knowledge, such data have been reported in

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only one study [175], where the role of the melatonin effects on Cx43 was evaluated with a heptanol test, whose outcomes did not differ in the melatonin-treated and control animals. It suggests that the influence of melatonin on the Cx43 expression, topology and phosphorylation state should be interpreted cautiously.

Another potential target for melatonin, which may be involved in the melatonin-related enhancement of conduction is a function of sodium channels in the cardiomyocytes. Indeed, our experiments with a week-long melatonin treatment [175] demonstrated the increase in the peak amplitude of sodium current density in the treated rats, which was associated with the acceleration of conduction and the enhancement of the expression of Nav1.5 channels at the levels of mRNA transcripts and protein. Forming of sodium channels is under control of regulation influences. Recent studies showed that sodium channel expression can be enhanced by persistent protein kinase A activation [176]. This pathway is less probable to be involved in the melatonin-dependent increase in SCN5A expression since the major effect of melatonin mediated by Gi protein is the downregulation of the protein kinase A-dependent cascade [103]. However, this mechanism should not be totally excluded since MT1 receptor could be also coupled with Gs protein and thus lead to the increase in cAMP production and activation of protein kinase A [108]. On the other hand, the enhancement of SCN5A expression can be mediated by Akt/ERK1/2-FOXO1 signalling pathway [177-179], which is subject to melatonin activation [105, 106]. Anyway, the functionally relevant enhancement of the sodium channel expression is a novel effect of melatonin, which deserves serious attention.

5.2.2. Conduction Velocity (Acute Effects)

In acute experiments in a porcine acute myocardial ischemia model, it was found that melatonin infusion immediately after the onset of coronary occlusion resulted in the shortening of the activation times in the border zone [157]. This effect was expressed in the mitigation of the QRS prolongation during first minutes of ischemia [163]. The activation enhancement by melatonin was the only change to be associated with the melatonin-related decrease in VT/VF incidence in this model. This effect was related only to the so-called phase 1A ischemic arrhythmias, which develop during first minutes of myocardial ischemia. In pigs, this phase lasts approximately 5-7 minutes, whereas in humans its duration might be longer due to sevenfold slower progression of myocardial infarction in the human heart as compared to the pig heart [180]. The fact that melatonin effect was limited to the phase 1A can be ascribed to distinctions of arrhythmogenic mechanisms or alternatively to the short half-life of melatonin in case of intravenous administration.

At the cellular level in the rat right ventricular preparations, melatonin application did not change the action potential upstroke velocity, but leads to a more complete restoration of the RMP at reoxygenation after an episode of hypoxia [155]. This effect suggests a completely different mechanism of the effect of melatonin on conduction in acute settings based on maintaining relatively high availability of sodium channels by limiting the inactivation process.

It was found [123] that melatonin application prevents ischemia-induced slowing of conduction velocity, and this effect was abolished by luzindole. Luzindole alone being applied in the nonischemic conditions decreased the longitudinal but not transverse component of conduction velocity, which implies direct or indirect involvement of sodium channels and/or Cx43, which are predominantly localized at the polar regions of cardiomyocytes. In patch-clamp experiments, melatonin alone did not produce any changes in the transmembrane potentials or ionic currents, while luzindole significantly decreased the inward rectifier potassium current (IK1) and caused RMP depolarization. A combined application of luzindole and melatonin resulted in intermediate changes implying that melatonin partly compensated the action of luzindole. Apart from the effects on IK1 and RMP, luzindole decreased Cx43 phosphorylation and expression, whereas melatonin produced opposite effects [123]. In other studies in the acute settings, the application of melatonin increases phosphorylation of Cx43 [181, 161, 123] and prevents its lateralization [181]. Similarly, the acute effects of melatonin on Cx43 are reduced by the application of the nonselective receptor blocker luzindole [123, 181] and selective MT2 receptor blocker 4-PPDOT [161].

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The effects of melatonin receptor blockers imply the role of receptor-dependent signaling, specifically MT2 and/or MT1/MT2 heteromers in the acute conduction-promoting action of melatonin. Moreover, the fact that the effects of luzindole could be more pronounced than the effects of melatonin itself [123] suggests that melatonin receptors exhibit constitutive activity previously demonstrated in other cells and models [111-114]. The signalling cascade linking MT1 and/or MT2 receptors with the electrophysiological end-targets remains still not fully understood. PKC is involved in MT1 and MT1/MT2-dependent signalling [115, 116, 103], and specifically PKCε has been proved to participate in Cx43 phosphorylation at Ser368 [182, 123]. Both MT1 and MT2 receptors decrease cAMP production via Gi protein transduction chain, while luzindole confers the opposite effect [103]. An increased cAMP level and activation of PKA influenced the Kir channels and decreases their activities in the cardiomyocytes [183]. Therefore, it can be suggested that melatonin receptors either constitutively active or activated by melatonin can increase the IK1 current flowing through the Kir channels, which maintains and/or restores RMP in the ischemic or reperfused myocardium, respectively. The fact that luzindole application increases VT/VF incidence and abolished the acute antiarrhythmic effects of melatonin [123] underlines the importance of the constitutive or inducible receptor activity for arrhythmia vulnerability in the ischemic or postischemic conditions.

5.2.3. Duration and Dispersion of Repolarization

The effects of melatonin on APD might differ. In acute application in the isolated hearts, melatonin caused APD shortening [149] or prevented its prolongation by hypokalemia [181] when given at reperfusion or in the nonischemic conditions, respectively. Luzindole abolished the melatonin-related prevention of APD prolongation in the hypokalemic perfusion, which corresponds to the previously discussed relationship between melatonin receptor state and IK1 current, which is suppressed in low extracellular potassium level [184]. On the other hand, melatonin pretreatment immediately before ischemia induced in the isolated hearts [148] and in vivo [185] enhanced restoration of APD at reperfusion after the ischemic episode. Such differences might be based on different mechanisms and/or electrophysiological targets for melatonin in normal and ischemic conditions. It could be speculated that the ATP-dependent potassium current, which is absent in the nonischemic myocardium but dramatically augmented at ischemia might be affected by melatonin. However, this suggestion has to be tested directly.

The changes in APD caused by melatonin are also expressed in the reduction of DOR. While DOR consistently increased in the ischemic conditions, the acute melatonin application alleviates these changes in ischemia and/or reperfusion in the rat [123], rabbit [185] and porcine [163] experimental models. The latter effect was evident from the observation of the shortened Tpeak-Tend intervals in the electrocardiogram of the melatonin-treated pigs. Increased DOR can serve as a prerequisite for reentry tachyarrhythmias, and acute application of melatonin indeed attenuated arrhythmogenesis in rats [123] and pigs [163]. However, no associations between the changes in DOR and VT/VF incidence were found in these two models. A similar absence of such associations was observed in the model with chronic melatonin treatment [155].

The mechanism of APD and DOR changes is currently unclear, probably except the cases when the luzindole-sensitive changes in repolarization duration could be ascribed to the changes in IK1 (see above). In acute application, luzindole did not modify DOR in ischemia-reperfusion model [123]. It possibly reflects the fact that in the ischemic conditions the changes in IK1 contribute little, if any, to the changes in DOR driven largely by the changes in IK(ATP). In chronic treatment, the melatonin influences on the DOR were associated with its influences on the activity of superoxide dismutase [155]. This observation might mean a mere coincidence, but also might reflect the effect of melatonin as an antioxidant on ROS-dependent arrhythmogenic mechanisms discussed above. Anyway, although melatonin has been demonstrated to decrease DOR and reduce the incidence of VT/VF in the same experimental models, these two effects were not associated with each other. It implies that the documented antiarrhythmic effect of melatonin is accounted for by the melatonin-related changes other than those in DOR.

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5.2.4. Melatonin Effects on Extrasystolic Activity

Suppression of extrasystolic activity was the first documented antiarrhythmic effect of melatonin [142]. This effect can have at least several plausible explanations; however, none of them have direct confirmations thus far. As melatonin can influence APD (see above), it can change the probability of the development of early and delayed afterdepolarizations. Since depolarization of RMP in the ischemic region facilitates extrasystolic activity [25], the ability of the active melatonin receptors to maintain RMP [123, 155] could result in the decrease in the extrasystolic burden. Also, extrasystolic activity can be augmented under the overactivation of the sympathetic system [30, 31, 186]; and melatonin, on the other hand, is known for its systemic [187] and cardiac [188] sympathoinhibitory effects, which might cause the decrease in the incidence of PVB. However, such a relationship was not demonstrated in the ischemia-reperfusion model in rats chronically treated with melatonin [189].

PVBs are not necessarily present a problem per se, it is important that they can trigger life-threatening arrhythmias such as VT/VF. It means that the effects of melatonin on the VT/VF incidence might be the consequence of its possible action on extrasystolic burden. However, in spite of that melatonin could induce “parallel” changes in the incidence of PVB and VT/VF [143, 156], these data could not be interpreted as that one was converted into the other. Furthermore, our recent studies demonstrated that melatonin reduced the VT/VF incidence but did not affect the extrasystolic burden [163, 162, 155]. Probably, the relationship between melatonin effects on these two characteristics is complex and might depend on the properties of the model used (chronic vs acute melatonin treatment, in vivo vs in vitro experiments, ischemia vs reperfusion arrhythmias).

6. Perspectives and Conclusions

Thus, melatonin has been demonstrated in many studies as a cardioprotective agent acting via its signalling pathways and by exercising its antioxidative properties. This multifaceted protection includes antiarrhythmic effects, which manifest in different conditions, specifically under myocardial ischemia or ischemia-reperfusion. The antiarrhythmic properties of melatonin may reflect its general protective action, which is responsible for the limitation of myocardial injury and inflammation. However, we intended to provide evidence that melatonin confers specific electrophysiological effects by targeting cardiomyocyte ion channels. These specific electrophysiological changes induced by melatonin signalling modify cellular and tissue properties in a way, which renders myocardium less arrhythmogenic. These specific changes operate either independently or together with general protective effects. One example of such a combination is the improvement of conduction by enhancing sodium current, Cx43 function and by limiting the fibrosis resulting from chronic inflammation.

We also intended to show that in spite of the fact that in the context of arrhythmia prevention many properties of melatonin could be potentially favorable not all of them appear crucial for the antiarrhythmic effects. Melatonin can limit myocardial injury by several mechanisms including ROS scavenging and enhancing cellular antioxidant systems. It in turn can minimize inexcitable regions constituting a core of reentry. Melatonin can reduce DOR, a prerequisite for the unipolar conduction block. By different mechanisms, it can also mitigate extrasystolic activity, which triggers reentry. However, the most important parameter, which is modified by melatonin and appears critical for arrhythmogenesis, at least in myocardial ischemia (or reperfusion), is conduction velocity. Maintaining conduction in the ischemic myocardium by melatonin is associated with the reduction in the number of life-threatening arrhythmias. This major role of melatonin-related enhancement of conduction velocity in the development of the antiarrhythmic properties manifests both in acute and chronic treatments with melatonin. However, the mechanisms of conduction enhancement appear different.

The long-term effect of melatonin treatment results in the activation of expression of sodium channel protein mediated probably by the Akt/ERK1/2-FOXO1 signalling pathway. Utilization of this mechanism can be considered for the potential application not only for the prophylaxis of arrhythmias in the context of myocardial ischemia, but also in channelopathies (or other types of

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myocardial electrical remodeling) characterized by the insufficient function of sodium channels, such as Brugada syndrome. However, new research is warranted to further explore cellular mechanisms of this effect and to confirm that melatonin can normalize compromised expression of Nav1.5. Moreover, the mechanism of sodium channel regulation by melatonin might be involved in the disorders related to melatonin deficiency, since such conditions promote arrhythmogenesis [190, 191], which might be accounted for by insufficient formation of sodium channels.

The short-term (acute) antiarrhythmic action of melatonin could be related to its ability to promote the IK1 current responsible for maintaining the RMP. This effect can limit the impairment of conduction in the ischemic myocardium and probability of reentry. The potential limitation of this action could be related to the short half-life period of the acutely administered melatonin. However, the obtained data suggest that regulation of the Kir channels responsible for the IK1 current largely relies on the constitutive activity of the melatoninergic pathways. It raises a question, if it is possible and effective to maintain such kind of melatonin signalling activity to prevent potential arrhythmic complications in susceptible patients.

Here, we summarized the data concerning the antiarrhythmic effects of melatonin. It could be suggested that these effects could be largely ascribed to the novel melatonin-related mechanism regulating myocardial conduction velocity. The functional relevance of this effect warrants its further exploration concerning cellular mechanisms, efficiency and limitations.

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