During healing after myocardial infarction (MI), the necrotic area within heart muscle is replaced by connective tissue, which forms a scar that restores the integrity and tensile strength of the healed organ. The scar developed after healing is characterised by a stable collagen network linked with glycosaminoglycans (GAG) and other extracellular proteins and a low number of myofibroblasts situated within the matrix. GAGs are repeating disaccharide units composed of hexosamine and uronic acid. After attachment to the protein core they form proteoglycans. GAGs regulate the water/electrolyte balance in the extracellular space, determine the action of growth factors (1) and enzymes and possibly also exert an influence on gene expression (2). Fomovsky et al. (3) suppose that proteoglycans are involved in controlling myocardial mechanics. The compounds regulate water content in the tissue and may influence the volume and stiffness of the heart. The knockout animals for versican, aggrecan or perlecan die prenatally or postnatally. Malformations of the heart found in autopsy are supposed to occur due to collagen fibrillogenesis disturbances (3). In patients affected with mucopolysaccharidoses cardiomiopathy was connected with accumulation of GAG. The mechanics of the heart was improved by enzyme replacement therapy. Thus, excessive accumulation of GAG is supposed to play an important role in the pathogenesis of cardiomyopathy (4).

Decreased myocardial reperfusion injury and reduced infarct size were found in desulfated heparin treated dogs. The effect of GAG was connected with anti-inflammatory action comprising impairment of neutrophil influx to the area of infarction (5). Beneficial effects on rat heart repair with hialuronan mixed esters of butyric and retinoic acid have also been proved (6).

Recruited fibroblasts synthesise extracellular matrix components, which remains under the regulatory influence of the endocrine system (7-9). The inflammatory factors have also a regulatory influence on the extracellular matrix formed in the wound (10). Inflammation has been implicated in the pathogenesis of heart failure (HF) and a role for chemokines in...
the pathogenesis of myocardial remodelling and development of HF has also been hypothesized. Other processes initiated after inflammation include cell proliferation, migration and protein synthesis.

As part of the inflammatory process, reactive oxygen species (ROS) are formed in the ischemic area. Oxidative stress is an important regulator of expression of chemokines and cell adhesion molecules, which are critical protein factors in the recruitment of leukocytes to sites of inflammation and injury. Hence, ROS may augment the inflammatory reaction by stimulating either cytokine release or induction of adhesive molecule expression (11, 12). ROS by themselves may also injure cells within the healing area (13) and accordingly some beneficial effects of antioxidative therapy on subjects affected by myocardial infarction, have been described (14).

Lately we have found that the accumulation of collagen in the infarcted heart scar is influenced by the pineal secretion product, melatonin (8, 15). Thus, physiological doses of melatonin increased the collagen level in the scar in a dose-dependent way while pinealectomy had an opposite effect. Moreover, pineal indoleamine supplementation of the pinealectomized rats normalized the collagen level in the heart (8). A 60 µg/100 g b.w. dose of melatonin fully reversed the effect of pinealectomy in the scar: this dose of melatonin expressed an equivalent effect to endogenously-secreted pineal indoleamine (8).

Melatonin is also reported to be a free radical scavenger and stimulator of antioxidative enzyme activity (16-18). The pineal indoleamine reduces the organ damage in ischemia/reperfusion injury (19) and ionizing radiation damage (20). In the two processes the free radicals are considered to be responsible for cell injury.

In light of the existing data melatonin is shown to be a cardioprotective factor (19) which may involve both direct and indirect antioxidant properties of pineal indoleamine (21). Thus, clinical trials have been proposed for melatonin treatment of patients with cardiovascular diseases (21, 22). Although, the effects of physiological doses of the pineal indoleamine (8, 15) on the collagen content of a heart subjected to myocardial infarction have already been described, the role of pharmacological doses of melatonin in heart repair remains to be elucidated. In our study we aimed at defining the effect of pharmacological doses of melatonin on the extracellular matrix composition (glycosaminoglycans and collagen content) in the infarcted heart scar. To fully elucidate the mechanism of the changes induced by melatonin, it is important to know whether the effects described are dependent on the direct influence of pineal indoleamine on myofibroblasts within the scar.

MATERIAL AND METHODS

Animals and experimental design

Male Wistar rats (66 in total) weighing 300±30 g were housed with free access to commercial food pellets (LSM, Bacutol, Poland), and tap water ad libitum. The animals were kept in light (L)-dark (D) conditions of 12:12. The light was turned on at 7:00 in the morning. The study was approved by the Local Commission of Ethics.

In the first part of the study, rats were randomly divided into 5 groups of 12 animals each. On day 1 of the experiment, myocardial infarction (MI) was induced in each rat by ligation of the left coronary artery.

Group 1: served as control (rats with induced MI and not treated with melatonin or vehicle).

Group 2: rats received only the vehicle on a different schedule. Group 2 was additionally divided into 2 subgroups of 6 animals. Animals in the first subgroup received the vehicle twice a day: between 5:00 and 6:00 in the afternoon, while the rats in the second subgroup received the vehicle twice a day: between 7:00 and 8:00 in the morning and between 5:00 and 6:00 in the afternoon. Vehicle (3% ethanol diluted in 0.9% NaCl) was administered intraperitoneally at a volume of 0.5 ml/100 g b.w.

Group 3: rats were administered with 300 µg/100 g b.w. melatonin (Sigma, St. Louis, USA) once a day: between 5:00 and 6:00 in the afternoon.

Group 4: rats were injected with 3 mg/100 g b.w. melatonin once a day: between 5:00 and 6:00 in the afternoon.

Group 5: rats were injected twice a day with 1.5 mg/100 g b.w. melatonin between 8:00 and 9:00 in the morning and additionally between 5:00 and 6:00 in the afternoon.

The animals were injected intraperitoneally with either vehicle or melatonin after the left coronary artery ligature, during the following 28 days of healing.

Six rats in each group were examined for the level of oxidative stress on day 7 of the experiment since this is a critical time for regulation of the extracellular matrix synthesis by inflammatory factors; at this point the inflammatory process is still present, fibroblasts are accumulating in the area of infarction and synthesis of extracellular matrix by the wounded tissue is beginning. The remaining 6 rats in each group were euthanized on day 28 of the experiment and used for the measurement of GAG and collagen content in the heart.

In the second part of the study, on day 28 of the experiment, the MI scars were aseptically removed from six additional rats with induced MI and used afterwards for the cell culture.

Left coronary artery ligation

Myocardial infarction in all rats was induced by ligation of the left coronary artery according to Seyle’s method with some modifications (23). After pentobarbital anesthesia (50 mg/kg b.w.), rats were ventilated with positive pressure through a tube inserted into the trachea and attached to a small animal respirator. After opening the pericardium, the heart was brought outside and the left coronary artery was ligated with nylon suture 6/0. The ligation was performed in the middle of the imaginary line between the left margin of the pulmonary cone and the closest point of insertion of the left auricular appendage. The heart was removed, and the left ventricle, right ventricle, scar and septum were separated and weighed. In all samples, total collagen and GAG content were estimated. In the experiment, 2 animals died (one from the intact control and second from the melatonin-treated group – 300 µg/100 g b.w.). The animals of all groups were in good condition. Symptoms of left-sided heart failure were not seen in any groups in autopsiey. Melatonin treatment did not influence the weight of the scar.

Cell culture

The MI scars were aseptically removed from the hearts and stored in RPMI 1640 medium containing gentamycin (25 µg/ml) and fungizone (2.5 µg/ml). Scars were minced and incubated in a 0.1% collagenase solution (37°C, 5% CO2) for 3 hours. The tissues were then centrifuged (5 min, 1000 rpm) and the supernatants discarded; the cell pellets were washed with DMEM containing 10% fetal calf serum, gentamycin (25 µg/ml) and fungizone (2.5 µg/ml), centrifuged (5 min, 1000 rpm) and plated on dishes. After 2 hours, the non-adherent cells were washed out. The adherent cells were cultured in a CO2 incubator at 37°C, under a 100% humidified atmosphere of 5% CO2 and 95% air. The cells were grown to confluence and then
trypsinized and passaged to new flasks. The new cell culture was set up at the initial cell density of 8 x 10^4/cm², based on the count of the trypan blue-stained cells in the Burkner chamber. The experiments were carried out on cells at the second or the third passages. The tested cells were grown in DMEM containing 3% calf serum, antibiotics at concentrations given above and passages. The tested cells were grown in DMEM containing 3% fetal bovine serum and 10% SDS (250 µl/well) and the absorbance (A0) was measured at 412 nm. Ellman’s reagent (1 mM 5,5'-dithio-bis-nitrobenzoic acid, DTNB) was added afterwards and the samples were incubated at 20°C for 20 min. In order to remove the excess of chloramine T, 1.25 ml of 3.15 M perchloric acid was added. After 5 min, the samples were treated with 1 ml of 20% p-dimethylaminobenzaldehyde and incubated in a 60°C water bath for 20 min. The optical density was measured at 560 nm on a spectrophotometer.

**Determination of protein concentration**

Protein concentration was estimated by the Lowry method (26) using bovine serum albumin as a standard.

**Determination of collagen**

The collagen was estimated according to the method described by Woessner (27). For evaluation of total collagen, the macerated tissue was extracted with ether-acetone and vacuum-dried at 90°C. Samples of the total collagen were assayed for hydroxyproline by hydrolysis with 6 N HCl (3 ml/10 mg of dry tissue) at 110°C for 24 hours. Hydrolyzates were evaporated to dryness in a water bath and precipitates were dissolved in 3 ml of redistilled water. The samples were neutralized with 1 N NaOH and diluted to 10 ml with redistilled water. Samples of 0.2 ml were taken for further analysis and diluted with redistilled water to the final volume of 2 ml. Hydroxyproline was oxidized to pyrrole by 1.25 ml of chloramine T in a citrate buffer (pH=6.0), then shaken for 5 min and incubated at 20°C for 20 min. In order to remove the excess of chloramine T, 1.25 ml of 3.15 M perchloric acid was added. After 5 min, the samples were treated with 1 ml of 20% p-dimethylaminobenzaldehyde and incubated in a 60°C water bath for 20 min. The optical density was measured at 560 nm on a spectrophotometer.

**Determination of glycosaminoglycans**

The 1,9-dimethylmethylene blue (DMMB) assay of Farndale and co-workers (28) was used for the estimation of GAG in the samples. Briefly, the samples were homogenized, defatted with an ether-acetone mixture (3:1) and the residue was dried to constant weight at 90°C. Fifty milligrams of dry sample was added to the mixture composed of 0.75 M NaOH and 50 mM natrium borate and incubated for 1 hour in a water bath at 73°C. After incubation, the pH was neutralized with 6 M HCl to approximately 7.0 and then 72 µl of 100% TCA was added to each sample to precipitate proteins. After centrifugation (6000 rpm for 30 min), 6 ml of 100% ethanol was added to the supernatant. To precipitate GAG, the samples were put into a bath for 20 min. The precipitate containing GAG was dissolved in 0.2 ml of distilled water.

Finally, 1.2 ml of DMMB-reagent (Aldrich Chemical Co) was added to 50 µl of the sample and the absorbance was measured at 525 nm on a spectrophotometer. DMMB reagent is composed of 51 mM DMBB, 45 mM glycine, and 41 mM NaCl adjusted to pH 3.0 with 1 M HCl.

**Statistical analysis**

The Kruskal-Wallis’s test was used for statistical analysis. Statistical differences between the groups were evaluated by the U Mann-Whitney’s test. The minimal level of significance was p<0.05.

**Preparation of myocardial infarction scar homogenates**

Myocardial infarction (MI) scars were immediately removed, washed with physiological saline, frozen by solid CO₂ and stored at -80°C until further analysis. A 10% homogenate was prepared in 1.15% KCl (1 g of tissue and 10 ml of KCl). Homogenates were centrifuged for 10 min at 3 000 x g and the supernatants were used for biochemical analysis of oxidative stress.

**Estimation of total thiol group content**

Total thiol group content was determined with 5,5’-dithiobis-2-nitrobenzoic acid (DTNB) according to the method of Ellman (24). Five µl of supernatant was transferred to a 96-well plate containing 10 mM sodium phosphate buffer (pH 8.0) and 10% SDS (250 µl/well) and the absorbance (A0) was measured at 412 nm. Ellman’s reagent (1 mM 5,5’-dithio-bis-nitrobenzoic acid, DTNB) was added afterwards and the samples were incubated at 37°C for 1 hour. The absorbance (A1) measurement at 412 nm was repeated and the difference between the two values (A1-A0) was calculated. The content of the sulfhydryl groups was estimated from the standard curve for reduced glutathione, which was used as standard for the range of concentrations between 0-1 mM. The concentration of the ~SH group was calculated as nmol/mg protein.

**Determination of reduced glutathione level**

Reduced glutathione was analyzed by the method of Ellman (24) using a 96-well microplate assay. Supernatants from homogenated scars were deproteinized by adding trichloroacetic acid (TCA) to a final concentration of 2.5%. The protein precipitate was removed by centrifugation (600 x g, 10 min). Both the 0.5 M sodium-phosphate buffer (pH=8.0) and 5 mM Ellman’s reagent were added to the appropriate wells and mixed with 5 µl of supernatants. The samples were incubated for 30 min at room temperature and the absorbance was measured at 412 nm against blind samples (reagents). The concentration of GSH in the samples was estimated on the basis of the standard curve, prepared for the reduced glutathione in a 0–1 mM range of concentrations.

**Estimation of lipid peroxidation**

Lipid peroxidation was assessed with the fluorescent probe C11 BODIPY 581/591 (4, 4-difluoro-5-(4-phenyl-1, 3-buta dienyl)-4-bora-3a, 4a diaza-s-indacene-3-indacenoic acid, Invitrogen, Carlsbad, CA, USA) according to the method of Aldini et al. (25). The probe is a fluorescent fatty acid analogue with a maximum of light emission within the red range (λex=530 nm/λem=590 nm). Upon free-radical oxidation (mainly by superoxides) this maximum is moved to the green range (λex=485 nm, λem=510 nm). The supernatants of homogenized and centrifuged MI scars were therefore diluted with PBS in a 1:5 ratio and the fluorescence intensity was measured with two sets (A and B) of wavelengths: A (red): λex=530 nm/λem=590 nm (F1) and B (green): λex=485 nm/λem=510 nm (F2) without a probe (blank). Then C11 BODIPY 581/591 was added to a final concentration of 1 µmol/l, the samples were incubated in the dark for 10 min at room temperature and the fluorescence intensity was measured again under the same conditions – F1 (A) and F2 (B). The results were presented as a ratio of F1/F2 – F1 fluorescence intensity.

**Determination of collagen**

The collagen was estimated according to the method described by Woesner (27). For evaluation of total collagen, the macerated tissue was extracted with ether-acetone and vacuum-dried at 90°C. Samples of the total collagen were assayed for hydroxyproline by hydrolysis with 6 N HCl (3 ml/10 mg of dry tissue) at 110°C for 24 hours. Hydrolyzates were evaporated to dryness in a water bath and precipitates were dissolved in 3 ml of redistilled water. The samples were neutralized with 1 N NaOH and diluted to 10 ml with redistilled water. Samples of 0.2 ml were taken for further analysis and diluted with redistilled water to the final volume of 2 ml. Hydroxyproline was oxidized to pyrrole by 1.25 ml of chloramine T in a citrate buffer (pH=6.0), then shaken for 5 min and incubated at 20°C for 20 min. In order to remove the excess of chloramine T, 1.25 ml of 3.15 M perchloric acid was added. After 5 min, the samples were treated with 1 ml of 20% p-dimethylaminobenzaldehyde and incubated in a 60°C water bath for 20 min. The optical density was measured at 560 nm on a spectrophotometer.

**Statistical analysis**

The Kruskal-Wallis’s test was used for statistical analysis. Statistical differences between the groups were evaluated by the U Mann-Whitney’s test. The minimal level of significance was p<0.05.
RESULTS

The obtained scars were thin and hard and usually constituted 40%-60% of the left ventricle free wall.

The GAG content in the scar after myocardial infarction was 5-6 times higher than the GAG content within the muscle of the viable part of the left ventricle, septum or right ventricle (p<0.001, Fig. 1A).

The results of rats injected i.p. once (subgroup 1 of group 2) or twice (subgroup 2 of group 2) a day with the vehicle were comparable and were presented as one bar. Thus, we conclude that vehicle treatment did not influence the GAG level in the MI scar compared with untreated control. The total GAG level was markedly decreased in both groups of rats: those treated with 0.3 mg melatonin/100 g b.w. (p<0.05), and treated with a 10-fold higher concentration – 3 mg melatonin/100 g b.w. (p<0.01).

Moreover, GAG content in the scar was also lowered in animals receiving the same daily dose of melatonin (3 mg/100 g b.w.), but split into two separate doses of 1.5 mg/100 g b.w. (p<0.01, Fig. 1B). Collagen content was not influenced by pharmacological doses of melatonin (Fig. 1C).

Ultrastructural findings (Fig. 2). The shape of the cells observed under the electron microscope was typical of fibroblasts. They were spindle-shaped, long and thin with oval nuclei demonstrating morphological features characteristic of high metabolic activity. They predominantly contained euchromatin, but a thin marginal zone of heterochromatin, situated close to the nuclear envelope, was also visible. The cell nuclei were a spongy-like type with vastly granular and fibrilar components, indicating high transcriptional activity. The cytoplasmic organelles were, in general, typical for fibroblasts. The rough endoplasmic reticulum was well developed and showed marked dilatations. The distinct Golgi zone had dilated dictiosomes and numerous vacuoles and vesicles. Fascicles of thin fibrils (Fig. 2, arrows) were seen close the cellular membrane, constituting a continuous bunch along the whole length of one side of the cell. This structure suggests the presence of actin fibers in the cell cytoplasm. We observed small vesicles (Fig. 2, arrows head), localized...
between the cell membrane and actin bunches which were closely connected with the cell membrane and sometimes opened to the outer space. They can be recognized as caveolae, responsible for calcium ion storage. The presence of actin bunches and caveolae connected with the cell membrane suggests that the observed cells are myofibroblasts, a differentiated form of fibroblasts.

Treatment of the myofibroblast cell cultures with 0.001% DMSO did not influence the GAG level compared with untreated control cells. An increased level of GAG was found in cell cultures treated with 10^{-7} M melatonin compared with the untreated control cells. An increased level of GAG was found in DMSO did not influence the GAG level compared with differentiated form of fibroblasts. 

suggests that the observed cells are myofibroblasts, a responsible for calcium ion storage. The presence of actin between the cell membrane and actin bunches which were

Fig. 4. Peroxidation of lipids (C11 BODIPY 581/591 lipid probe) by homogenates of the infarcted heart scar (A). The results were presented as a fluorescence ratio of red (\(\lambda_{em}=530\) nm/\(\lambda_{em}=590\) nm) and green (\(\lambda_{em}=485\) nm/\(\lambda_{em}=510\) nm) fluorescence intensity of the probe. The content of total sulphhydril groups (B) and the content of reduced glutathione (C) in the infarcted heart scar. The above results refers the infarcted heart scar in the controls; group of vehicle treated rats consisting of 2 subgroups: subgroup 1- intraperitoneal injection of 3% solution of ethanol diluted in 0.9% NaCl once daily (between 17.00-18.00) and subgroup 2- injected with the same solution twice daily (between 8.00-9.00 and additionally between 17.00-18.00); groups of rats injected with melatonin (MLT) at doses of 0.3 mg/100 g b.w., 3 mg/100 g b.w. once daily (17.00-18.00) and 1.5 mg/100 g b.w. twice daily (8.00-9.00 and additionally between 17.00-18.00). Each bar represents the mean ±S.D. of five-six samples.

DISCUSSION

The present study shows a marked elevation of GAG in the MI scar compared with the viable part of the left ventricle, septum and the right ventricle (Fig. 1A). This phenomenon was noted four weeks after myocardial infarction and suggests an increased level of GAG during scar formation. This data is in agreement with our previous results showing the transient elevation of GAG in the scar six weeks after myocardial infarction (23). Melatonin decreased GAG level in the MI scar at all applied doses (300 µg/100 g b.w., 3 mg/100 g b.w., 2 x 1.5 mg/100 g b.w., Fig. 1B), while in myofibroblast cultures, the opposite effect, namely an increase in the GAG content, was found for the 10^{-7} M dose of pineal indoleamine. Lower concentrations of melatonin (10^{-4}M-10^{-6}M) did not change the GAG amount in the cultures (Fig. 3), which suggests that the effect, obtained in vivo, is not dependent on the direct action of pharmacological doses of melatonin on myofibroblasts in the scar. (Figs. 1B and 3). Some data indicates an influence of the pineal indoleamine on the function of the endocrine, nervous and immune systems (29-31). Thus, melatonin induced changes of the regulatory systems may influence the GAG content. However, the myofibroblasts in the cultures were not subjected to hemodynamic stress that could modify their synthetic activity. Melatonin applied at pharmacological doses does not influence collagen content (Fig. 1C), but rather reduces GAG level. Thus, changes of the collagen glycosaminoglycans assembly in the extracellular space are postulated. The present data is in agreement with the previous report (8) showing that only a narrow range of doses 30-60 µg/100 g b.w. is effective. The lower dose (3 µg/100 g b.w.) or higher dose (300 µg/100 g b.w.) did not influence the collagen content in the MI scar.

In the present study, melatonin was found to attenuate the oxidation of the lipid probe by MI scar tissue (Fig. 4A). This phenomenon reflects the reduction of oxidative stress in MI scars and shows that the pineal indoleamine may stabilize cell membranes and protect them from oxidative damage. These changes are accompanied by an elevation of the content of protein and glutathione sulphhydril groups (Fig. 4B and 4C). In our study

Lipid peroxidation was shown as the ratio of reduced probe (red spectrum of fluorescence, \(\lambda_{em}=530\) nm, \(\lambda_{em}=590\) nm) to oxidized probe (green spectrum of fluorescence, \(\lambda_{em}=485\) nm, \(\lambda_{em}=510\) nm). Melatonin applied at 3 mg/100 g b.w. (p<0.05) and 2 x 1.5 mg/100 g b.w. (p<0.05) increased the ratio of reduced to oxidized probe, which suggests that the pineal indoleamine at a dose of 3 mg/100 g b.w. reduced oxidation of lipids. The pineal indoleamine at a dose of 300 µg/100 g b.w. was not effective. The level of lipid peroxidation in this case was the same as in control samples (Fig. 4A).

An increased amount of total sulphhydril groups in the scar after myocardial infarction was found in rats receiving a daily dose of melatonin: 300 µg/100 g b.w. (p=0.025), 3 mg/100 g b.w. (p<0.001) or 2 x 1.5 mg/100 g b.w. (p=0.003) (Fig. 4B). All these doses of melatonin also elevated the level of the reduced glutathione in the heart MI scar (Fig. 4C). Both the total content of the sulphhydril groups and the glutathione level were similar in control groups. The total –SH content (in nmol/mg protein) increased by 6.17±3.79 (MLT 300), 22.1±3.67 (MLT 3) and 13.25±2.18 (MLT 2x1.5). An increase in sulphhydril groups of glutathione (in nmol/mg protein) in comparable groups were 3.73±1.35 (MLT 300), 4.76±1.04 (MLT 3) and 2.96±0.67 (MLT 2x1.5). Thus, the obtained data suggests that an increase of the total –SH groups reflects mainly the protein oxidation status.
the level of protein sulphhydryl groups was in equilibrium with that of reduced glutathione. This is in accordance with the results by Goraca et al. (32, 33), who observed parallel changes in the protein and glutathione –SH group content in heart tissue subjected to oxidative stress. Glutathione keeps the thiol groups of protein in a reduced state, and in doing so, stabilizes their structure and determines their functions. In our experimental model, an elevation of the total sulphhydryl group content was only slightly dependent on an increase in the level of reduced glutathione. Since the total amount of protein and collagen did not change in our experimental conditions we can assume an increased level of –SH groups to be a biomarker of diminished protein oxidation (34). Melatonin was reported to increase glutathione synthesis in the brain. The pineal indoleamine stimulates the activity of γ-glutamylcysteine synthase, which is the rate-limiting enzyme of glutathione synthesis (34). The reduced glutathione level could be also dependent on melatonin and the activity of both glutathione peroxidase and reductase (35). Myocardial infarction leads to generation of oxygen free radicals and reduction of antioxidant defence (36). Thus, long-lasting melatonin application reverses this phenomenon and restores the level of antioxidants. In the infarcted area, oxygen free radicals increase inflammation via modification of cytokine release (11). The present study shows that the pineal indoleamine may reduce the inflammatory reaction and change the mediator level, which could influence the GAG content in the scar. The inflammatory mediators were proved to have an influence on the repair process (10).

Several studies have reported antioxidative effects of melatonin in the rat heart (14, 19, 37, 38). Reduced infarct volume in hearts subjected to ischemia/reperfusion injury has been observed after treatment with melatonin at doses of 1 or 10 mg/kg b.w. (14). The indoleamine injections at pharmacological doses before ischemia or during reperfusion decreased the ventricular fibrillation number and arrhythmia score (37). Melatonin administered as a bolus injection in a broad range of doses (0.5–5 mg/kg) before occlusion of the coronary artery decreased premature ventricular contractions, ventricular tachycardia and fibrillation. Moreover, the pineal indoleamine was reported to reduce infiltration of neutrophiles into the ischemic area and decrease superoxide anion production (38). This data, however, was obtained with the ischemia-reperfusion model, where a single bolus injection of melatonin was made. In the present study, rats were injected once or twice daily and the coronary blood vessel, which was occluded by surgery, was not reperfused.

The present study confirms that melatonin applied at pharmacological doses reduces the level of GAG in the scar of the infarcted heart. Since the mechanism of GAG reduction cannot be explained by the direct action of the pineal indoleamine on the myofibroblasts in the scar, we hypothesise that changes of GAG could be caused by indirect melatonin effects. This paper also documents the antioxidative role of melatonin in the infarcted area of the heart.

Summarizing, this is the first study to report the antioxidative effect of melatonin in the healing of myocardial infarction in rats subjected to long-lasting treatment with pineal indoleamine. Moreover, our work proves that long-lasting treatment with pharmacological doses of melatonin reduces oxidative stress and increases total antioxidant capacity in the infarcted tissue of rats affected by myocardial infarction. The hemodynamic effects of pharmacological doses of melatonin in myocardial infarction affected rats should be further investigated.

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