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Agomelatine-induced responses in rat aorta

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Abstract – Agomelatine is an agonist of the melatonergic MT1/MT2 receptors and an antagonist of the serotonergic 5-HT receptors. Its actions mimic melatonin in antioxidative and anti-inflammation. However, the vascular effects of agomelatine has not been investigated. In this study, we investigated the responsiveness of agomelatine on rat thoracic aorta. Cumulative addition of agomelatine (10-8-10-3 M)-induced relaxation against serotonin (5-HT, 10-6 M)-induced tone in rat aorta with and without endothelium. In different preparations the effects of nitric oxide synthase inhibitor NG nitro-L-arginine methyl esther (L-NAME, 10-4M), Ca2+-activated K+ channel blocker tetraethylammonium (TEA, 10-3M) or nonselective phosphodiesterase inhibitor 3-Isobutyl-1-methylxanthine Isobutyl-1-methylxanthine (IBMX, 10-6M) were investigated on agomelatine-induced responses. Agomelatine produced concentration-dependent relaxations of rat thoracic aorta with endothelium pre-contracted with 5-HT. Pre-incubation with L-NAME, or endothelium denudation decreased the relaxant response. In the presence of TEA, the concentration-response curve to agomelatine shifted to the right, however maximal relaxation was not significantly different from the control. Pre-treatment with IBMX increased the sensitivity to agomelatine significantly. The results indicate that nitric oxide, Ca2+-activated K+ channels and also phosphodiesterase played role in agomelatine-induced relaxation.

Keywords – Agomelatine, Nitric oxide, Phosphodiesterase, Rat aorta, TEA

1. Introduction

Agomelatine (N-[2-(7-Methoxy-1-naphthyl) ethyl]acetamide) is an unique analogue of melatonin (N-acetyl-5-methoxytryptamine). It acts synergistically on both the melatonergic (M1/M2 agonist) and serotonergic systems (5-HT2B and 5-HT2C receptor antagonist) [1], without influencing serotonin release; this activity results in the subsequent release of norepinephrine and dopamine. Although agomelatine has expected chronobiotic effects, it also has clinically significant antidepressant [2, 3] and anxiolytic [4] properties. These psychotropic effects have been proposed to be caused by the synergy between the melatonin-based and mono amine-based effects.

Agomelatine is a melatonin analogue and melatonin has protective effects on smooth muscle dysfunction. In vitro studies have shown that melatonin decreased vascular tone of vascular beds from control, hypertensive or aged animals, through the reduction of adrenergic contraction and the increase in acetylcholine-induced relaxation. It was reported that melatonin directly affects blood vessels by producing either relaxant responses in rabbit basilar artery [5], rat aorta [6,7], and sheep pulmonary artery and vein [8] or vasoconstriction in isolated caudal arteries from juvenile rats [9]. Furthermore, Weekly [10] reported that melatonin caused a dose-dependent relaxation of pre-contracted rat aortic smooth muscle that was not affected by the removal of vascular endothelium.

There is an increasing trend for antidepressant medication use nowadays. Although some adverse-effects of antidepressants could be related to a direct action on smooth muscle, the in vitro effects of agomelatine on vascular smooth muscle have not been investigated. The present study is the first demonstration of functional changes in the vascular smooth muscle of rat thoracic aorta to agomelatine. Therefore, the purpose of the present study was to determine the effects of agomelatine on serotonin-induced responses of rat thoracic aorta, paying special attention to the roles of the endothelial nitric oxide, Ca2+-activated K+ channels and also phosphodiesterase activity in these effects.

2. Materials and Methods

2.1. Animals

For this study, prior permission for animal experimentation was obtained from the Necmettin Erbakan University Experimental Medicine Research and Application Center Ethics Committee. Male Wistar rats, aged 3-4 weeks old, were obtained from Necmettin Erbakan University Experimental Medicine Research and Application Centre (Konya, Turkey). In this investigation, healthy adult male Wistar rats, 4 months of age and weighting 170-190 g were used. The animals were obtained from Necmettin Erbakan University, Experimental Research Centre, Konya, Turkey. The rats were housed in wire-topped opaque polycarbonate cages and maintained under constant environmental conditions with a 12 h light/dark schedule. The environmental temperature was $20 \pm 2\ ^{o}C$ and humidity was 50%.

2.2. Experimental design

Rats were sacrificed by cervical dislocations. The descending thoracic aorta was quickly isolated, cleaned and sectioned into 3- to 4-mm-long rings. The rings were then placed in organ baths containing Krebs–Henseleit

solution (KHS, mM: NaCl 119, KCl 4.70, MgSO4 1.50, KH2PO4 1.20, CaCl2 2.50, NaHCO3 25, Glucose 11), which were thermoregulated at 37 ºC and aerated (95% O_2 and 5% CO_2). Changes in isometric tension of aorting rings were recorded using a four-channel force-displacement transducer (BIOPAC MP36, Santa Barbara, Caslifornia, USA) connected through amplifiers to a ITBS08 Integrated Tissue Bath System (Commat, Ankara, Turkey). Endothelium intact rings were used in the first part of the study and the endothelium was removed by gently rubbing the internal surface of the vessels in another part of the study. Successful removal of the endothelium was confirmed by demonstrating the inability of acetylcholine (ACh) to induce relaxation. Rings were contracted with 5-HT (10^{-6} M) and then cumulative ACh (10^{-9}-$3x10^{-4}$ M) was applied for the control of endothelium. Then, the rings were washed and rested, to evaluate the effect of agomelatine on vascular tension, rat aorta rings with endothelium from six different preparations were exposed to 5-HT (10^{-6} M) to induce constriction. Agomelatine (10^{-8}-10^{-3} M) was added after the maximal vasoconstrictive response to 5-HT had been achieved. To determine whether the relaxant effect of agomelatine is dependent on the endothelium, rat aorta rings without endothelium were studied in another part of the investigation. As agomelatine induced a relaxant effect in the presence of endothelium, the concentration-response curves were obtained after treatment with the nitric oxide synthase inhibitor N^G nitro-L-arginine methyl esther (L-NAME, 10^{-4} M). L-NAME had been added to the organ bath 20 min before agomelatine concentration response curves were obtained. To determine the role of Ca^{2+}-activated K^+ channels and also phosphodiesterase activity on agomelatine-induced relaxations, aortic rings precontracted with 5-HT were tested in the presence of tetraethyl ammonium; a Ca^{2+}-activated K^+ channel blocker (TEA,10^{-3} M) or 3-isobutyl-1-methylxanthine (IBMX,10^{-6} M) in different preparations.

2.4. Statistical Analysis

Relaxation responses to agomelatine were expressed as percentages of the 5-HT (10^{-6} M) induced contraction. Concentrations of agomelatine causing 50 % of the maximal response (IC_{50}) were calculated from each individual concentration-response curves. Maximal responses (E_{max}) and pIC_{50} (-log IC_{50}) values for curves obtained before and and after L-NAME, TEA and IBMX incubation were compared by using Student's t test. Statistical significance was set at $p < 0.05$.

2.5. Drugs

Serotonin chloride, N^G nitro-L-arginine methlyl esther, acetylcholine chloride, tetraethyl ammonium (all dissolved in distilled water), agomelatine, 3-isobutyl-1-methylxanthine (dissolved in dimethyl sulphoxide; DMSO). All drugs were obtained from Sigma (St. Louis, MO, USA).

3. Results and Discussion

5-HT (10-6 M) produced reproducible contractions in rat thoracic aorta rings. After the maximal contractile response to 5-HT, cumulative addition of agomelatine (10-8- 10-3 M) caused concentration-dependent relaxations (Figure 1). The values of pIC50 and maximum effect (Emaz) of agomelatine are given in Table I. The relaxant effect of agomelatine was significantly reduced by removal of the endothelium from the aorta sections (n=6, $p < 0.05$). L-NAME (10-4 M) treatment also significantly suppressed the vasorelaxant effect of agomelatine (n=6, $p < 0.05$). The effects of agomelatine on aortic rings were investigated after inhibition of PDE activity by IBMX (10-6 M), a nonselective PDE inhibitor and in the presence of Ca2+-activated K+ channel blocker (TEA,10-3 M). No significant difference in maximum relaxation induced by agomelatine was found between the preparations with or without IBMX or TEA treatment. Compared with control agomelatine response, the sensitivity was significantly enhanced in the presence of IBMX. Furthermore, as seen in Table I, pre-treatment with TEA significantly decreased the sensitivity to agomelatine (n=6, $p < 0.05$).

This study, demonstrated that agomelatine-induced relaxation was mediated via nitric oxide, Ca2+-activated K+ channels opening and phosphodiesterase inhibition activities in rat thoracic aorta. Agomelatine is an antidepressant with an unique receptor profile as a MT1/MT2 melatonergic agonist and 5-HT2C receptor antagonist [14]. Melatonin regulates vascular tone by interacting with specific receptors that are present in mammalian arteries. Three distinct melatonin receptor subtypes, termed MT1, MT2, and MT3 receptors, have been identified and shown to mediate the physiological effects of melatonin [6]. In the rat aorta, only MT1 melatonin receptor was found [15]. To our knowledge, there are no studies that analyze the effects of agomelatine on the rat aorta.

Table I: pD2 (- log IC50) and Emax (% maximum relaxation) values for agomelatine in the with (E+, control), without endothelium (E-) and in the presence of L-NAME (10-4M), IBMX (10-6M), TEA (10-3 M).

	pIC_{50}	E_{max}
Control	5.52 ± 0.01	100 ± 0.0
L-NAME	-	40.0 ± 2.0*
E (-)	-	38.0 ± 3.0*
TEA	4.52 ± 0.15*	100 ± 0.0
IBMX	6.40 ± .0.20*	100 ± 0.0

Values are mean ± SD. Each value is derived from six experiments.*Statistically significant ($p < 0.05$) compared with control value.

In this study, no direct effect of agomelatine was observed on resting tone, cumulative addition of agomelatine caused concentration-dependent relaxations of pre-contracted rat thoracic aorta rings with endothelium. In blood vessels, the endothelium plays an important role in regulating vascular smooth muscle tone by releasing relaxing and contracting factors [13], nitric oxide is a major factor in the cardiovascular system and believed to account for most of the endothelium-derived relaxing factor activity released within the vessel wall. In this study, to determine if vascular responses were mediated by an intact endothelium especially nitric oxide, dose-response studies for agomelatine were repeated in the

presence of L-NAME and also in endothelium denuded rings. Both denudation of vessel endothelium by mechanically or preincubation of thoracic aorta rings with L-NAME (10-4 M), reduced the responses to agomelatine significantly. The relaxant effect of agomelatine in endothelium denuded rings was not significantly different from that in L-NAME pre-treated rings. This suggest that endothelial nitric oxide is important for agomelatine-induced relaxation in rat aorta. Currently, it is generally accepted that endothelium-derived factors, such as nitric oxide, have an important role in modulating relaxation in the rat aorta. Similarly, Satake et al [14] reported that the vasorelaxing effect of melatonin (10−6–10-3 M) on the 5-HT response is endothelium dependent in rat aorta. In this study, we observed relaxations at lower concentrations than 10−6 M, with agomelatine.

On the other hand, several mechanisms are involved in vasorelaxation other than endothelial factors, such as K+ channel opening and phosphodiesterase pathway inhibition [15]. Agents those increasing both cAMP and cGMP, inhibiting phosphodiesterase and opening K+ channels are able to relax agonist-induced vasoconstriction more fully [16]. K+ channel activation is one of the important mechanisms to promote vasorelaxation. Thus, we investigated whether Ca2+-activated K+ channel would be involved in the relaxant effect of agomelatine. Pre-incubatation with TEA a Ca2+-activated K+ channel blocker [17] of aortic rings with intact endothelium, significantly reduced the vasorelaxation to agomelatine, confirms the participation of Ca2+-activated K+ channels in relaxation induced by agomelatine. In a previous study, we observed that low concentrations of TEA (<3 mM) are effective at blocking large conductance Ca2+-activated K+ channels [18].

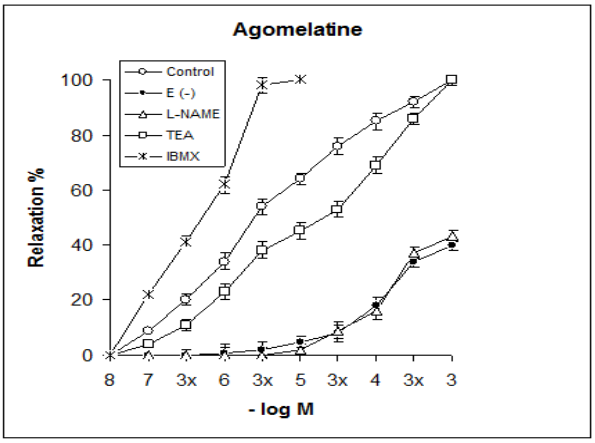

Figure 1: Concentration-response curves showing relaxations induced by agomelatine (10-8- 10-3M) in rat thoracic aorta with (E+ ; control) and without (E-) endothelium, in the presence of L-NAME (10-4M); IBMX (10-6M); TEA (10-3M). Each point represents the mean ± SD expressed as percentage of the tension developed by 10-6M 5-HT. Each value is derived from six experiments

Intracellular second messengers cAMP and cGMP are important players in the modulation of smooth muscle tone under physiological and pathophysiological states. They can reduce the calcium sensitivity of the contractile proteins, resulting in relaxation, termed calcium desensitization Phosphodiesterases are widely distributed in mammalian tissues and hydrolyze cAMP and cGMP, [19]. These substances able to raise intracellular levels of cAMP or cGMP show a strong relaxant effect, which can be due to phosphodiesterase inhibition [20]. In this study, the relaxant potency of agomelatine was increased in the presence of a nonselective phosphodiesterase inhibitor IBMX. Rodríguez-Ramos et al [21] reported that IBMX is capable of increasing the levels of cAMP and cGMP and of inducing relaxation of vascular smooth muscle tissue.

4. Conclusion

In this work, the mechanism underlying the vasorelaxant action of agomelatine in rat aorta was elucidated for the first time. The removal of endothelium or pre-treatment of intact aortic ring with L-NAME reduces significantly the vasorelaxant response of agomelatine. The relaxation response to agomelatine is possibly modulated by endothelial nitric oxide.Inhibitions of Ca2+-activated K+ channels in aortic rings with TEA decrease the sensitivity to agomelatine. Ca2+-activated K+ channels are involved and morever phosphodiesterase isoenzymes play an important role as modulators of rat aortic smooth muscle.

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