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Melatonin analogue agomelatine reduces rabbit's intraocular pressure in normotensive and hypertensive conditions

Alejandro Martínez-Águila [a], Begoña Fonseca [a], Antonio Bergua [b], Jesús Pintor [a,*]

[a] Dpto de Bioquímica y Biología Molecular IV, Facultad de Óptica y Optometría, Universidad Complutense de Madrid, Madrid, Spain
[b] Department of Ophthalmology, University of Erlangen-Nuremberg, Erlangen, Germany

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ABSTRACT

In the search for new compounds to reduce intraocular pressure (IOP), with fewer side effects, we have found that agomelatine, a melatonin analogue, can reduce IOP being, therefore, interesting for the treatment of ocular hypertension and glaucoma. In normotensive conditions, agomelatine (10 μl 100 μM) reduced IOP by 20.8 ± 1.4% (n=18) with a maximal effect 180 min after the compound application and 68.8 ± 5.7% (n=8) in a hypertensive condition. Concentration–response curve depicted a sigmoid behaviour presenting a pD2 value of 9.7 ± 0.3 which was equivalent to an EC_{50} of 0.19 nM. The effect of agomelatine was partially antagonized by 4PPDOT (MT_2 antagonist receptor. 10 μl 100 μM) and prazosin (MT_3 antagonist receptor. 10 μl 100 μM) (85.6 ± 1.6% and 87.2 ± 1.9%, N=18 respectively.) Agomelatine hypotensive effect in normotensive condition was comparable to latanoprost (40 μl) and brimonidine (40 μl) and it was no so effective as dorzolamide (40 μl) or timolol (40 μl). These results may suggest the use of this melatonin analogue for the treatment of those ocular conditions, which involve an abnormal raise of intraocular pressure.

1. Introduction

Glaucoma is a heterogeneous group of eye diseases leading to pathological alterations of the optic nerve head. This neuropathy is accompanied by a chronically elevated intraocular pressure (IOP), causing irreversible blindness (Bergua et al., 2009). Elevated IOP is one of the main risk factor in the developing of glaucoma. If the pressure is high or it maintains slightly higher for a long period of time, apart from the damage in the ciliary arteries, there is a mechanical damage of the optic nerve head, which also can contribute to the impairment of visual field or blindness (Brusini and Johnson, 2007).

There are drugs that can modify intraocular pressure (IOP) because they are able to reduce the production of the aqueous humour from the ciliary processes. Also some substances can increase the rate of filtration of aqueous humour through the trabecular-meshwork or uveoscleral pathways. These drugs are included in different groups such as parasimpathomimetics, alpha2-agonists, beta-blockers, carbonic anhydrase inhibitors and prostaglandins analogues. Although all of them reduce IOP, none are exempt of side effects like blurred vision, tachycardia or arrhythmia (Higginbotham et al., 2002; Kaiserman et al., 2009; Quigley, 1993).

In the search for new compounds for the treatment of glaucoma, new products have been tested, both natural and synthetic. One of the natural products tested in its ability to reduce IOP is melatonin (Alarma-Estrany et al., 2011).

Melatonin (5-methoxy-*N*-acetyltryptamine) is a neurohormone secreted by the pineal gland, which has a circadian rhythm in its production and secretion to the blood flow. Its levels in blood rises up during the evening and it is maximum at 2 in the morning (Lincoln et al., 1981). Melatonin has been related to many important aspects of the medical investigation. Therapeutic potential of this substance has been demonstrated in the treatment of ocular, blood and gastrointestinal tract diseases, diabetes mellitus, rheumatoid arthritis, fibromyalgia, infectious diseases, sleep disturbances, aging and depression (Sanchez-Barcelo et al., 2010).

Many authors have demonstrated the presence of melatonin in the eye and in particular in intraocular tissues (Quay, 1984). Contradictory reports indicate that this neurohormone can raise IOP (Meyer-Bothling et al., 1993), or reduce it (Osborne, 1994). In this sense, Osborne suggests that the main part of the controversy is due to two factors, the way of application of melatonin and the different types of animals in which melatonin has been tested (Osborne, 1994).

A promising melatonin analogue is agomelatine (S-20098; Valdoxan®, Servier), which is claimed as a potent agonist of the MT_1 and MT_2 melatonin receptors (Conway et al., 2000), and a non-selective inhibitor of the 5-HT_{2C} receptor (Millan et al., 2003).

* Corresponding author. Tel.: +34 913946859; fax: +34 913946885.
E-mail address: jpintor@vet.ucm.es (J. Pintor).

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Agomelatine is nowadays used for the treatment of depression (De Berardis et al., 2011). However, little is known about the effect of agomelatine on intraocular pressure. Since some melatonin analogues produce an ocular hypotensive effect (Alarma-Estrany et al., 2011), the present experimental work describes the effect of this molecule on IOP in New Zealand white rabbits both in normotensive and in a hypertensive condition.

2. Methods

2.1. Animals

New Zealand white rabbits, weighting 3–4 kg, were used for IOP studies. The animals were kept in individual cages with free access to food and water. They were submitted to control 12 h/12 h light/dark cycles. All the protocols herein comply with the ARVO Statement for the Use of Animals in Ophthalmology and Vision Research and also are in accordance with the European Communities Council Directive (86/609/EEC).

2.2. Intraocular pressure measurements

Agomelatine (Santa Cruz Biotechnology, USA) was formulated in isotonic saline containing 1% DMSO (Sigma, St. Louis, USA) and tested at different concentrations from 10^{-12} M to 10^{-4} M and was applied in drops to the cornea at a fixed volume of 10 μl in the treated eye. The contralateral eye received the same volume of saline +1% DMSO. IOP was measured by means of a TonoVet® contact tonometer supplied by Tiolat Oy (Finland).

In order to study the effect of agomelatine, two IOP measurements were taken before agomelatine was instilled, after that twice during the first hour, and once every hour for 6 h.

Luzindole (a non-selective melatonin antagonist), prazosin (a MT_3 melatonin antagonist) and 4-phenyl-2- propionamidotetralin (4PPDOT, a MT_2 melatonin antagonist) were used as antagonists of melatonin receptors. Due to the effect of prazosin in the α_1 receptor, corynanthine was applied to test the effect of inhibition only in that receptor.

In order to study the effect of these antagonists, they were instilled 10 μl 30 min before agomelatine at a concentration of 100 μM, measuring IOP in the same fashion as previously described.

The commercial compounds (latanoprost (Xalatan® 0.005%, Pfizer, Madrid, España), dorzolamide (Trusopt® 2%, Neurogard, Madrid, España), timolol (Timoftol® 0.5%, Frosst, Madrid, España) and brimonidine (Alphagan® 2 mg/ml, Allergan, Madrid, España)) were tested at a fixed volume of 40 μl.

2.3. Hypertensive condition

To induce the hypertensive condition, animals placed in Trendelenburg position (prone −80° head down) and IOP were monitored every 5 min for a maximal of 20 min. After this, animals were placed in horizontal and they rested for 2 h. Next, the selected compound was administered and animals were placed again in Trendelenburg position when the maximum effect of the compound was reached (normally 3 h after the application).

2.4. Statistical analysis

All data are presented as the mean ± S.E.M. Statistical differences between treatments were calculated using ANOVA test and t test. Plotting and fitting were carried out by GraphPad Prism 5 computer program (GraphPad Software).

3. Results

3.1. Effect of agomelatine on intraocular pressure in New Zealand white rabbits

In order to study the effect of agomelatine on intraocular pressure, a single dose of 100 μM (10 μl) was tested and the time-course was followed along 6 h. As it can be seen in Fig. 1, agomelatine produced a reduction in IOP when compared to controls (contralateral eye with the same volume of saline +1% DMSO). This effect was statistically significant 2 h after the instillation and lasted for 3 h until it returned to control values. The maximal reduction in IOP was 20.8 ± 1.4% ($n=18$. $P<0.001$, t-test) values, which was obtained 180 min after the application of the compound (Fig. 1).

3.2. Concentration–response behaviour for agomelatine on IOP

Agomelatine was tested in a broad range of concentrations starting from 10^{-12} M to 10^{-4} M in order to see its effect on IOP.

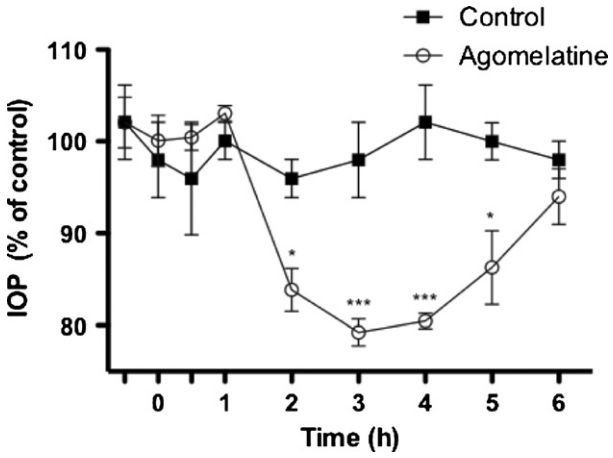

Fig. 1. Effect of agomelatine on rabbit intraocular pressure. Time course of agomelatine (100 μM, 10 μl) followed for 6 h. 100% represents the intraocular pressure before application of any drug (i.e., at t_0) and was equivalent to 17.2 ± 1.6 mm Hg. Values represent the mean ± S.E.M of 18 independent experiments. $^{*}P<0.05$ $^{***}P<0.001$, with respect to control levels (t-test).

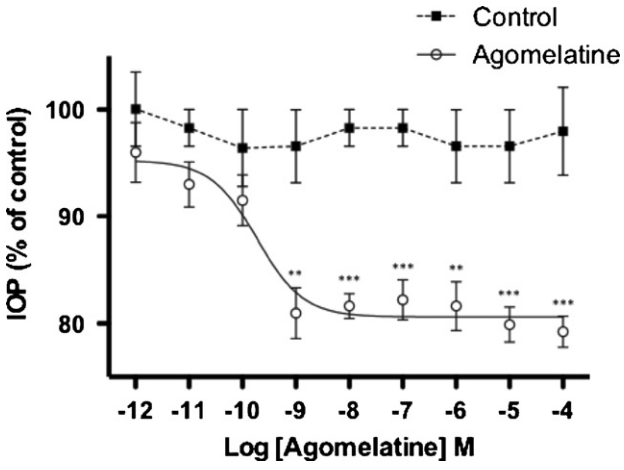

Fig. 2. Concentration–response course for agomelatine. Graded doses of agomelatine were applied as described in methods. The maximal reduction in intraocular pressure due to agomelatine was 79.2 ± 1.4%. Values represent the mean ± S.E.M of six independent experiments. $^{*}P<0.05$ $^{**}P<0.01$, with respect to control levels (Two-way ANOVA test with Bonferroni posttests).