ELSEVIER

European Journal of Pharmacology 416 (2001) 251–254

ejp

www.elsevier.nl/locate/ejphar

Short communication

Involvement of melatonin MT_3 receptors in the regulation of intraocular pressure in rabbits

Jesús Pintor [a,*], Laura Martin [a], Teresa Pelaez [a], Charles H.V. Hoyle [b], Assumpta Peral [c]

[a] *Departamento de Bioquímica, E.U. Óptica, Universidad Complutense de Madrid, c / Arcos de Jalón s / n, 28037 Madrid, Spain*
[b] *Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK*
[c] *Departamento de Óptica, E.U. Óptica, Universidad Complutense de Madrid, c / Arcos de Jalón s / n, 28037 Madrid, Spain*

Received in revised form 16 February 2001; accepted 20 February 2001

Abstract

Melatonin, a neurohormone secreted by the pineal gland, can stimulate three subtypes of receptors, namely: mt_1, MT_2 and MT_3. We examined the ability of melatonin and the selective MT_3 receptor agonist, 5-methoxycarbonylamino-*N*-acetyltryptamine (5-MCA-NAT), to modify intraocular pressure in rabbits. Both compounds significantly reduced intraocular pressure, maximally by 24% and 43%, respectively, with IC_{50} values of 363 ± 23.0 and 423 ± 30.0 ng/10 μl (1.6 ± 0.1 and 1.8 ± 0.1 nmol, respectively). The non-specific melatonin receptor antagonist, luzindole, abolished the depressant effect of both compounds, thus confirming the involvement of melatonin receptors. Our results show, for the first time, a functional response that may be regulated by melatonin MT_3 receptors, and provide evidence that supports a role of melatonin in the circadian changes of intraocular pressure.

Keywords: Intraocular pressure; Melatonin; Melatonin MT_3 receptor; 5-MCA-MAT (5-methoxycarbonylamino-*N*-acetyltryptamine); GR 135531

1. Introduction

Melatonin, *N*-acetyl-5-metoxytryptamine, is a neurohormone produced during the night by the pineal gland, and which serves to regulate circadian rhythms, informing the organism about the photoperiod. So far, receptors for this compound have been divided into three subtypes: mt_1, MT_2 and MT_3 melatonin receptors (Dubocovich, 1995). The first two have been cloned and are negatively coupled to adenylate cyclase via a pertussis toxin-sensitive G-protein. The third type, MT_3 melatonin receptor, has not been yet cloned and seems to be coupled to phospholipase C (Mullins et al., 1997). Effects mediated via mt_1 and MT_2 have been reported in the central nervous system (CNS), cardiovascular system and reproductive organs (for review, see Brzezinski, 1997).

Little is know about the melatonin MT_3 receptor type. This receptor can be characterised by means of the high-affinity ligand, 5-methoxycarbonylamino-*N*-acetyltryptamine (5-MCA-NAT, also known as GR 135531) (Molinari et al., 1996). More recently, comprehensive binding studies have been performed (Paul et al., 1999). However, no physiological activity has yet been reported.

Intraocular pressure is higher during the daytime than at night, which suggests that it is regulated by effectors of circadian rhythms. Controversial results have been obtained regarding the modulation of intraocular pressure by melatonin in rabbits, mainly due to the diverse strains of animals and the methodology used for the measurements (Osborne, 1994).

We now describe, for the first time, an action of melatonin and 5-MCA-NAT mediated via a putative melatonin MT_3 receptor: a reduction in intraocular pressure in New Zealand white rabbits.

2. Materials and methods

2.1. Animals

New Zealand white rabbits (males, 2–3 kg) were used. The animals were kept in individual cages with free access to food and water, under controlled 12-h/12-h light/dark

* Corresponding author. Tel.: +34-91-394-3890/6859; fax: +34-91-394-3909.
E-mail address: jpintor@eucmos.sim.ucm.es (J. Pintor).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)00864-0

cycles. Two batches of eight rabbits were used. This protocol adheres to the Association for Research in Vision Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. Also, the experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC).

2.2. Intraocular pressure measurements

Intraocular pressure was measured by means of a TONOPEN contact tonometer supplied by MENTOR (USA). The experiments were performed following a masked design. When administering the compounds, no visible indication was given as to made of the corresponding solution (saline and compound). Agents were applied topically, unilaterally, to the cornea in volumes of 10 μl. The contralateral eye received the same volume of saline solution. Since the application of the tonometer may produce some discomfort in the animals, the corneas were anaesthetised by applying 10 μl of 1:10 (v:v) oxibuprocacaine/tetracaine (4 and 1 mg, respectively). Two measurements were taken 30 min apart before any substance was applied.

2.3. Pharmacological studies

Melatonin and 5-MCA-NAT were used for a dose–response curve. Doses ranging from 10 pg/10 μl to 1 mg/10 μl were applied (equivalent to 43 fmol–43 μmol and 34 fmol–34 μmol, for melatonin and 5-MCA-NAT, respectively) and intraocular pressure was measured at 0.5, 1, 2, 3, 4, 5 and 6 h after the application. On any one day, only a single dose was tested on a single animal. Animals were rested for at least 2 days between doses. Melatonin and 5-MCA-NAT, were prepared at a 10–100-fold higher concentration in dimethyl sulphoxide (DMSO). Then they were diluted (1:10 or 1:100) in saline solution the desired final concentration.

The non-specific melatonin antagonist, luzindole, was added 30 min before the application of either melatonin or 5-MCA-NAT compounds at a dose of 100 μg/10 μl (i.e. 342 nmol).

2.4. Statistical analysis

All data are presented as the means ± S.E.M. Significance of differences was determined by two-tailed Student's *t* test. Dose–response curves plotting and fitting were carried out with the computer programme Microcal Origin v.3.5 (Microcal Sofware USA).

2.5. Compounds

Melatonin was purchased form Sigma (St. Louis, MO). 5-MCA-NAT and luzindole were from Tocris (Bristol, UK). Oxibuprocacaine/tetracaine anaesthetic was from CUSI labs (Spain). Other reagents were analytical grade from Merk (Darmstadt, Germany).

3. Results

3.1. Effect of melatonin and 5-MCA-NAT on rabbit intraocular pressure

Melatonin and 5-MCA-NAT (10 pg/10 μl to 1 mg/10 μl) produced a dose-dependent decrease in intraocular pressure which was maximal at 10 μg/10 μl, with a reduction of 24.4 ± 4.4% ($n = 8$) (Fig. 1A). 5-MCA-NAT (10 pg/10 μl to 1 mg/10 μl) also produced a dose-dependent decrease in intraocular pressure, which was maximal at 100 μg/10 μl, with a reduction of 43.1 ± 3.65% ($n = 8$) (Fig. 1A). The maximum response to 5-MCA-NAT

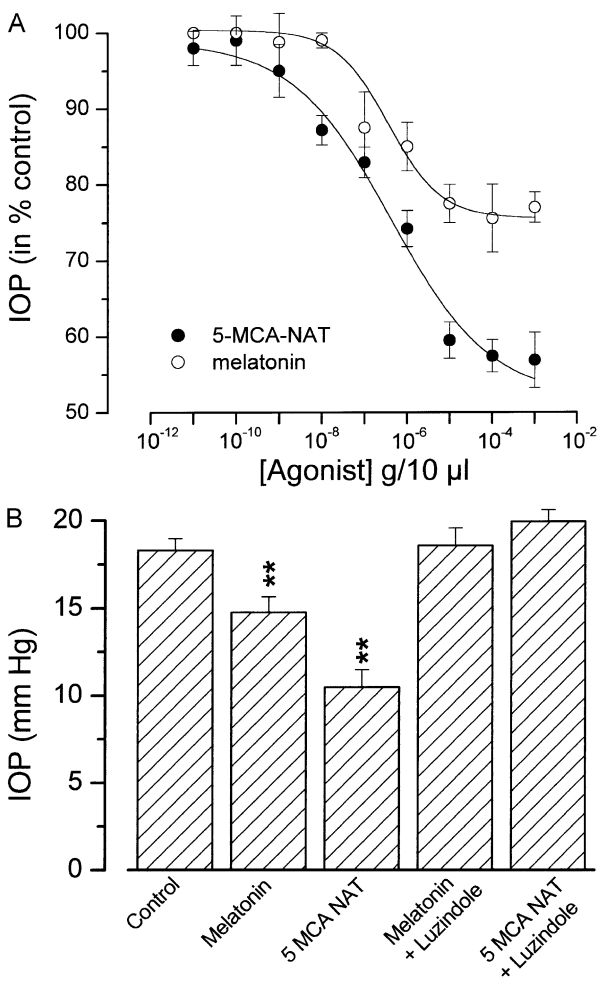

Fig. 1. Dose–response curves for melatonin and 5-methoxycarbonylamino-*N*-acetyltryptamine (5-MCA-NAT) and effect of antagonist, luzindole. (A) Dose–response curves for melatonin and 5-MCA-NAT at the time which corresponded to the maximal effect in each case. (B) Antagonism by luzindole (100 μg/10 μl) of the responses produced by melatonin and 5-MCA-NAT. Values are the mean ± S.E.M. of eight independent experiments. $^{**}P < 0.005$, with respect to control levels, Student's *t*-test.

was significantly greater than that to melatonin ($P < 0.01$). The IC_{50} values for melatonin and 5-MCA-NAT were 363 ± 23.0 and 423 ± 30.0 ng/10 μl, respectively, which is equivalent to doses of 1.6 ± 0.1 and 1.8 ± 0.1 nmol, respectively. These values are not significantly different from each other.

Pretreatment with the non-specific melatonin receptor antagonist, luzindole (100 μg/10 μl), abolished the effect of both melatonin and 5-MCA-NAT (Fig. 1B). When applied alone, luzindole did not produce any change in the intraocular pressure over a period of 6 h (data not shown).

Melatonin produced a relatively transient decrease in rabbit intraocular pressure which was maximal by 1 h after application and which returned to its control levels over the next 3 h (Fig. 2A). 5-MCA-NAT produced a more rapid response with a dramatic fall in intraocular pressure during the first half hour after application. The effect was also more sustained, lasting longer than 6 h (Fig. 2B), the intraocular pressure levels being below the normal pressure values even after 9 h (data not shown).

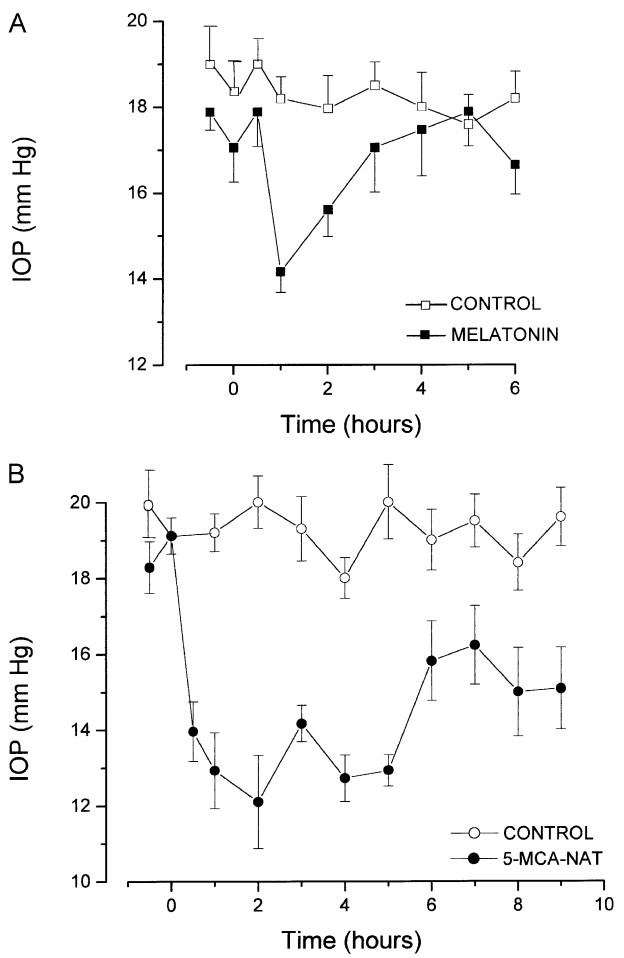

Fig. 2. Effect of melatonin and 5-methoxycarbonylamino-*N*-acetyltryptamine (5-MCA-NAT) on rabbit intraocular pressure. (A) Time course of melatonin (100 μg/10 μl) effect on rabbit intraocular pressure followed for 6 h from t_0. The first point ($t_{-0.5}$) was obtained in the absence of melatonin. Maximal effect of melatonin was reached at t_1. (B) Time course of 5-MCA-NAT (100 μg/10 μl) effect on rabbit intraocular pressure followed for 9 h from t_0. The first point ($t_{-0.5}$) was obtained in the absence of 5-MCA-NAT. Maximal effect of 5-MCA-NAT was reached at t_2. Points show the mean ± S.E.M. of eight independent experiments.

4. Discussion

The results showed that both melatonin and the selective MT_3-receptor agonist, 5-MCA-NAT, reduce intraocular pressure in a dose-dependent manner. Although the two compounds had similar IC_{50} values, 5-MCA-NAT was able to evoke a maximal response, almost twice that of melatonin. Assuming that there is a single population of melatonin receptors, melatonin seems to be behaving as a partial agonist whereas 5-MCA-NAT behaved as a full agonist. Other possibilities for explaining this behaviour should not be ruled out. The non-specific melatonin receptor antagonist, luzindole, abolished the responses to both agonists, suggesting that they are, indeed, working through melatonin receptors.

5-MCA-NAT has been used to characterise melatonin MT_3 receptors in binding studies, but no physiological function has been linked to this receptor (Molinari et al., 1996; Paul et al., 1999). This may be the first time that a functional action of melatonin or 5-MCA-NAT, mediated via the melatonin MT_3 receptor, has been reported. Possibly, the decrease in intraocular pressure evoked by melatonin in this experimental model represents a physiological activity related to the dynamics of aqueous humour, which is consistent with the nocturnal increased rate of synthesis of this hormone and the corresponding nocturnal reduction in intraocular pressure (Dubocovich et al., 1998). This is made the more likely, considering that elevated levels of melatonin are found during the night in the aqueous humour (Liu and Dacus, 1991).

Transduction mechanism and the location of melatonin MT_3 receptors in the anterior segment of the eye are yet not known. The ciliary processes and the trabecular meshwork are two possible locations since they are responsible for the synthesis and drainage of aqueous humour, respectively. Further work is necessary to elucidate whether either of the processes of production and resorption of aqueous humour is affected by activation of melatonin MT_3 receptors.

Melatonin MT_3 receptors are thought mediate to phospholipase C activation (Dubocovich, 1995; Mullins et al., 1997). Nevertheless, other possibilities cannot be excluded. For example, melatonin has been shown to evoke ionic currents in various ocular tissues in culture. One of these tissues is the trabecular meshwork in whose cells membrane hyperpolarisation by some agents can lead to relaxation, which would permit a higher rate of drainage (Rich et al., 1999).

In summary, melatonin and 5-MCA-NAT can activate melatonin MT_3 receptors that decrease intraocular pressure. This fact may be related to changes in secretion of

this neurohormone during the night and to the corresponding diurnal fluctuation in intraocular pressure. These results suggest a potential significance of the development of melatonin or melatonin receptor MT_3 agonists as a therapeutic agent for the treatment of eye disorders that involve raised intraocular pressure.

References

Brzezinski, A., 1997. Melatonin in humans. N. Engl. J. Med. 336, 186–195.

Dubocovich, M.L., 1995. Melatonin receptors: are there multiple subtypes? Trends Pharmacol. Sci. 16, 50–56.

Dubocovich, M.L., Yun, K., Al-Ghoul, W.M., Benloucif, S., Masana, M., 1998. Selective MT_2 melatonin receptor antagonists block melatonin-mediated phase advances of circadian rhythms. FASEB J. 12, 1211–1220.

Liu, J.H.K., Dacus, A.C., 1991. Endogenous hormonal changes and circadian elevation of intraocular pressure. Invest. Opthalmol. Visual Sci. 32 (3), 496–500.

Molinari, E.J., North, P.C., Dubocovich, M.L., 1996. 5-[^{125}I]Yodo-5-metoxy carbonylamino-*N*-acetyltryptamine: a selective ligand for the characterization of melatonin ML_2 binding sites. Eur. J. Pharmacol. 301, 159–168.

Mullins, U.L., Fernandes, P.B., Ellison, A.S., 1997. Melatonin agonists induce phosphoinositide hydrolysis in *Xenopus laevis* melanophores. Cell Signal 9, 169–173.

Osborne, N.N., 1994. Serotonin and melatonin in the iris/ciliary processes and their involvement in intraocular pressure. Acta Neurobiol. Exp. (Warsaw) 54, 57–64.

Paul, P., Lahaye, C., De la Grange, P., Nicolas, J.-P., Canet, E., Boutin, J.A., 1999. Characterization of 2-[^{125}I]iodomelatonin binding sites in Syrian hamster peripheral organs. J. Pharmacol. Exp. Ther. 290, 334–340.

Rich, A., Farrugia, G., Rae, J.L., 1999. Effects of melatonin on ionic currents in cultured ocular tissues. Am. J. Physiol.: Cell Physiol. 45, C923–C929.