ORIGINAL ARTICLES—Laboratory science

Mechanism related to reduction of intraocular pressure by melanocortins in rabbits

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Abstract

Aim—**To investigate whether the ocular hypotensive effect of alpha melanocyte stimulating hormone (MSH) is related to eicosanoids or cyclic AMP (cAMP).**

Methods—**Intraocular pressure (IOP) readings were taken at a similar time on the day before and after a single dose of topical MSH. Changes in the levels of prostaglandin E_2 (PGE_2) and prostacyclin in incubated iris ciliary body (ICB) explants were measured by specific radioimmunoassay (RIA). Incubated ICB explants were exposed to MSH or adrenaline (epinephrine) for a week. In addition, cAMP levels in the medium were determined following short term incubation using RIA.**

Results—**A significant dose related reduction in IOP was noted with topical MSH (mean (SD) maximal effect 4.5 (0.1) mm Hg (21%); $p<0.001$ v appropriate baseline) which persisted up to 6 hours ($p=0.05$). MSH treated ICB explants showed a 1.5-fold increase in PGE_2 and prostacyclin levels ($p<0.001$ for each parameter) while cAMP levels were increased twofold ($p<0.001$).**

Conclusions—**A single application of MSH caused a sustained dose related ocular hypotensive effect with no side effects. An increase in eicosanoid and cAMP levels following ICB exposure to MSH indicated their involvement in MSH induced ocular hypotension. MSH and its analogues might have clinical relevance as antiglaucoma drugs with fewer side effects because of their antiallergic and anti-inflammatory properties.**

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Alpha melanocyte stimulating hormone (MSH) is a basic tridecapeptide which originates from pro-opiomelanocortin (POMC) found in the pituitary,[1] brain,[2] skin,[3] and at other sites.[4] POMC is the precursor for a family of biologically active peptides called melanocortins which include alpha, beta and gamma MSH, lipotropins, endorphins, and adrenocorticotrophic hormone (ACTH). MSH, named after its effect on the pigmentation of amphibian skin, is involved in modulation of neuroprotection, anti-inflammatory activity,[5–7] and obesity regulation.[8] Its effect is receptor specific and is not duplicated by ACTH, ACTH fragments, or glucocorticosteroids.[9 10]

In the eye MSH has been identified as a constitutive component of the normal aqueous humour of humans, rabbits, and mice.[11 12] Its involvement in the regulation of intraocular pressure (IOP) was first noted in the 1960s and 1970s when high doses of the hormone were found to have a biphasic effect on IOP.[13–15]

Melanocortins act by the activation of G protein coupled receptors and production of cyclic AMP (cAMP).[16] Cyclic AMP modulates IOP reduction by adrenergic agonists[17 18] and prostaglandin derivatives such as latanoprost.[19 20] Enhancement of eicosanoid production by MSH treated ocular pigmented epithelium[21] is another possible role for melanocortins in the regulation on IOP. The activity of melanocortins in most organs is dose related so we have examined its role in IOP regulation using physiological doses,[22 23] unlike previous studies.[13–15 24–26]

In this study we have investigated whether MSH causes a sustained reduction in IOP following a single topical application of alpha MSH in normotensive pigmented rabbits.

Materials and methods

ANIMALS
Normotensive pigmented rabbits of either sex weighing 2–3.5 kg were housed in standard cages in a temperature controlled room with free access to water and food (Laboratory Rabbit Chow, Kalston Purina Co) and were exposed to a 12 hour light-dark cycle. Institutional guidelines regarding animal experimentation were followed. During IOP measurements the rabbits were kept conscious.

IRIS CILIARY BODY (ICB) SAMPLE PREPARATION
The cornea and anterior segment were removed and the ICB was pulled gently and maintained for 5 days in a 24 well culture well (Nunc, Roskilde, Denmark) containing DMEM and 10% calf serum, and supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), and glutamine (2 nM) at 37°C in a humidified atmosphere of 10% carbon dioxide in air.

Table 1 *Effects of melanocyte stimulating hormone (MSH) on intraocular pressure (IOP)*

Time after application (hours)	MSH (10^{-8} M)	MSH (10^{-9} M)	MSH (10^{-10} M)
0 (baseline)	24 (0.3)	23 (0.8)	24 (0.5)
1	23 (0.7); (4.2%)	23 (0.7); (0%)	24 (0.5); (0%)
2	19 (0.6); (21%)*	20 (0.5); (13%)*	23 (0.7); (4.2%)
4	20 (0.5); (17%)*	20 (0.5); (13%)*	20 (0.5); (17%)*
6	21 (0.4); (12.5%)*	22 (0.7); (10%)	23 (0.5); (4.2%)

Values are mean (SD) with % IOP reduction.
*$p<0.05$ compared with untreated baseline values of treated eyes at the same time.

PROSTAGLANDIN E_2 AND PROSTACYCLIN DETERMINATION

Samples of the medium were obtained 2, 5, and 7 days after the start of ICB incubation and processed as described previously[21] for determination of PGE_2 and 6-keto $PGF_{1\alpha}$ (the stable prostacyclin metabolite) using the appropriate radioimmunoassay (RIA) kits (Amersham NEN, USA).

CYCLIC AMP DETERMINATION

Medium samples were taken 2 days after starting incubation and cAMP was determined by RIA (Amersham NEN, USA).

MEASUREMENTS OF IOP

IOP was measured in conscious rabbits using a Digilab model 30R as described previously[27] after topical application of one drop of local anaesthetic (Localin-Benoxinate HCl 0.4%, Fischer Pharmaceuticals, Israel) diluted 1:3 with sterile saline. The exclusion criteria were a consistent difference in IOP between the two eyes of 2 mm Hg or more, irritability, or any sign of ocular irritation.

A baseline IOP curve was plotted for each animal 1 day before treatment at 09.00, 11.00, 12.00, 13.00, and 15.00. The following day the drugs were applied topically at 08.00 to the right eye only and IOP readings were then taken at both eyes at similar times to the baseline curve, thus avoiding individual and seasonal changes in IOP.[21 27]

DRUG PREPARATION

MSH acetate (Sigma Chemical Co, St Louis, MO, USA) was dissolved in sterile saline (0.9% NaCl). The stock solution containing 250 µg/ml (10^{-4} M) was stored at –20°C and diluted with phosphate buffered saline (PBS) for topical application according to the protocol described.

STUDY DESIGN

The study consisted of in vivo and in vitro experiments.

In vivo study

This was a randomised, double blind, single dose study. IOP readings were taken as

described. The drugs were bottled in an identical manner and coded; decoding took place only at completion of the statistical analysis.

The in vivo study involved four groups of animals: control untreated rabbits (group 1) and groups 2–4 treated with MSH in concentrations of 10^{-8}, 10^{-9}, and 10^{-10}, respectively. The baseline IOP curve was determined and the following day the drugs were applied topically at 08.00 to the right eye only and IOP readings were then taken in both eyes at similar times. The effect of the drug on the IOP was calculated in mm Hg and was also expressed as a percentage (% IOP) of baseline as follows:

% IOP = (baseline IOP* – treated IOP**)/ baseline IOP

where baseline IOP* indicates the value at a given time.

In vitro study

Long term incubation involved determination of PGE_2 and $PGF_{1\alpha}$ levels in the medium of ICB whole explants incubated with MSH at a final concentration of 10^{-8} (group 1) and incubated in adrenaline (epinephrine) 1% (group 2). Samples were obtained for eicosanoid measurements at 2, 3, and 7 days while medium sampling for cAMP was done on day 2 only.

Short term incubation explants lasted 90 minutes at 37°C in a shaking bath after which a sample was obtained for cAMP measurements in the following groups: group 1 (control; no drug added); group 2 (treated with forskolin); groups 3 and 4 (treated with MSH at final concentrations of 1 nM and 10 nM, respectively).

STATISTICAL ANALYSIS

IOP reduction was calculated by comparing the measured value in mm Hg at each time point in the treated eye with the corresponding baseline measurement before treatment. Comparison of the IOP values was performed by analysis of variance (ANOVA) and the Student's *t* test.

Results

EFFECT OF MSH ON INTRAOCULAR PRESSURE

A single application of MSH caused a dose related reduction in IOP (Table 1). The maximal hypotensive effect was seen 2 hours after application of 10^{-8} M MSH (mean (SD) reduction in IOP 4.5 (0.1) mm Hg; 21% of baseline) and this lowering effect persisted at 4 hours (17%) and at 6 hours ($p<0.001$ and $p=0.05$ compared with the corresponding baseline levels, respectively). The reduction of IOP with MSH in a concentration of 10^{-9} M was smaller than that achieved with 10^{-8} M (13% at 2 and 4 hours, $p<0.05$ for each) while MSH in a concentration of 10^{-10} M caused a transient reduction in IOP (Table 1). IOP changes in the contralateral eye treated with MSH were negligible, and no other side effects were noted.

EFFECT OF MSH ON EICOSANOID LEVELS IN ICB

The concentrations of both PGE_2 and 6-keto $PGF_{1\alpha}$ in the conditioned media of control ICB

Table 2 *Effect of melanocyte stimulating hormone (MSH) on eicosanoid production by iris ciliary body*

Time of incubation	PGE_2 (pg/mg)		6-Keto-$PGF_{2\alpha}$ (pg/mg)	
	Control	MSH (n=10)	Control	MSH (n=10)
Day 2	900 (50)	1550 (100)*	250 (15)	400 (20)*
Day 5	300 (40)	440 (15)*	75 (20)	130 (15)*
Day 7	220 (60)	330 (20)*	50 (10)	80 (15)*

*$p<0.05$.

Table 3   Adrenergic drug and prostaglandin E_2 (PGE$_2$) production by iris ciliary body (ICB)

Group	PGE$_2$ (pg/mg)
Control	980 (150)
Saline	1090 (200)
Adrenaline 1%	2470 (310)*
MSH	1550 (290)*

Sampling was done on day 2 of ICB incubation.
*p<0.05 v control group.

Table 4   Melanocyte stimulating hormone (MSH) and cyclic AMP (cAMP) in iris ciliary body

Group	cAMP (fmol/µg protein)
Control	11 (0.5)
Forskolin	27 (1.5)*
MSH (1 nM)	19 (3)*
MSH (10 nM)	21 (4)*

*p<0.05 compared with control.

explants decreased during the first 7 days in culture (Table 2). Eicosanoid levels in MSH treated ICB explants were significantly higher than in the corresponding untreated explants during the first week of culture (p<0.001 at days 2, 5, and 7 for each v corresponding control). In order to test whether the reduced response of cultured explants to MSH over time could be attributed to degenerative changes, the hormone was added on day 5 to incubated but untreated retinal explants. This resulted 2 days later in a 1.5 increase in PGE$_2$ levels (p<0.0025), similar to the average increase observed on day 7 in ICB explants treated with the hormone from the start of incubation (data not shown). Addition of adrenaline to the incubation medium containing ICB caused a 2.5 increase in PGE$_2$ levels in the medium (p<0.001 v control or saline groups) on day 2 (Table 3).

CYCLIC AMP AND ICB EXPLANT CULTURE

Cyclic AMP levels in the incubation medium containing MSH treated ICB were dose related, reaching maximal values which were twice as high as those in the control medium at a concentration of 10 nM (Table 4).

Discussion

The ocular effect of MSH, first studied in the 1960s, has a biphasic IOP pattern[13–15] but the hypotensive phase has been overlooked. This study is the first to demonstrate that a single application of MSH induces a significant dose related reduction in IOP in rabbits. The maximal MSH induced decrease in IOP averaged 21% below baseline 2 hours after application, and was still statistically significant 4 and 6 hours later. Changes in IOP in the contralateral eye treated with MSH were negligible, and no other side effects were noted.

The finding that the MSH induced ocular hypotensive effect is dose related is in accordance with the fact that the activity of the drug is dose dependent in the nervous system,[6] skin,[3] and as an anti-inflammatory agent.[5–7] In our study the MSH induced ocular hypotensive effect was achieved with doses which were in the range of normal MSH levels in the rabbit aqueous.[11] In most previous studies MSH was used in non-physiological doses[17 24–26 28]

causing flare and increased IOP.[13 15 17] Krakau's findings[13 15] on MSH induced IOP increase and flare were possibly caused by inaccurate methodology. Another group,[28] using MSH in higher doses, reported no ocular side effects. Likewise, Hernandez et al injected MSH intracamerally[29] at a dose one million times greater than normal aqueous values, using a traumatic process. In these extreme non-physiological conditions no reliable conclusions can be drawn about the effect of MSH on IOP or flare.

The dose related biphasic effect of MSH is reminiscent of the early data on the ocular biphasic effect of prostaglandins reported in the 1970s.[30 31] The intracameral injection of prostaglandins produced a dose related initial hypertensive phase associated with a transient protein leakage into the aqueous.

The regulatory effect of MSH in pregnant women has not been suggested although it is well established that the IOP is reduced in normotensive and ocular hypertensive pregnant women during the second trimester of pregnancy.[32–38] At this time plasma levels of MSH in the mother are higher than normal[36 37] owing to excessive production by the embryo and placenta.[37 38] Our results showing MSH induced reduction in IOP indicate that MSH might be the protagonist in inducing ocular hypotension in pregnant women.

The mechanism by which MSH exerts its ocular hypotensive effect is as yet unknown. We have shown that MSH induces cAMP production in vitro by ICB; cAMP is involved in modulating IOP reduction by adrenergic agonists[17 18] while adenosine agonists lower IOP.[18] Thus, cAMP production by ICB in eyes exposed to MSH might be one of the underlying mechanisms for the ocular hypotensive effect of MSH. This is in agreement with the fact that melanocortins act by activation of G protein coupled receptors and production of cAMP.[16] Our study also found an increase in eicosanoid production by incubated ICB over a long incubation period. Adrenaline caused a similar increase in PGE$_2$ levels, which is in accordance with the finding that adrenergic agents with ocular hypotensive activity act partially through endogenous eicosanoid. The ocular hypotensive effect of various eicosanoid analogues is well established and latanoprost is currently used as an antiglaucoma agent.[39] It therefore seems likely that the IOP reducing effect of MSH is also related to an increase in eicosanoid levels.

Activation of adrenergic receptors regulating permeability of the blood-aqueous barrier might also play a part in the hypotensive activity of MSH which requires intact β adrenergic receptor sites,[5 40] while the anti-inflammatory effect of centrally administered MSH is inhibited by the systemic injection of non-specific β adrenergic receptor blockers.[5]

In summary, we have shown that MSH at physiological doses induces a significant and sustained reduction in IOP in normotensive rabbits. The hypotensive effect seems to be attributable to activation by MSH of cAMP, its

second messenger, and enhanced eicosanoid production.

The mechanism of action of melanocortins in regulating IOP still has to be determined but it is probably multifactorial and may include a modulatory effect of MSH on the blood-aqueous barrier through adrenergic receptors.

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