Inhibitory effect of methysergide on calcitonin gene-related peptide-induced vasodilatation and ocular irritative changes in the rabbit

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1 Calcitonin gene-related peptide (CGRP) is involved in ocular neurogenic inflammation in the rabbit, causing vasodilatation in the anterior uvea, breakdown of the blood-aqueous barrier, increase in the intraocular pressure (IOP) and rise in the adenosine 3':5'-cyclic monophosphate (cyclic AMP) content in the aqueous humour. So far there is no means of preventing these CGRP-induced ocular effects.

2 In the present study, the effect of intravenous methysergide (1–10 mg kg⁻¹, b.w.) on CGRP-induced changes in the IOP, blood-aqueous barrier and cyclic AMP content in the aqueous humour was studied *in vivo*. The effect of methysergide on CGRP-induced vasodilatation both *in vivo* and *in vitro* was also investigated.

3 Methysergide decreased intraocular pressure but had only a transient effect on blood pressure. Methysergide decreased the regional blood flow in ocular tissues by 53–65%, but did not have such a vasoconstrictor effect in most extra-ocular tissues studied.

4 Methysergide inhibited CGRP-induced vasodilatation, increase in the IOP, breakdown of the blood-aqueous barrier and increase in the cyclic AMP content in the aqueous humour *in vivo*.

5 *In vitro*, methysergide alone did not have effects on the vascular tone in isolated ophthalmic artery of rabbit. However, it potentiated noradrenaline (NA)-induced contraction. There were no differences in the IC₅₀ values for CGRP on the NA-induced contraction in the presence and absence of methysergide, indicating that methysergide has no direct effect on the vasorelaxant effect of CGRP *in vitro*.

6 The present study demonstrates that in the rabbit eye methysergide inhibits CGRP-induced changes. One inhibitory mechanism of methysergide may be to enhance the effect of a vasoconstrictor (NA) to antagonize the vasodilator effect of CGRP. The present findings suggest that a methysergide-sensitive mechanism may be used to limit some pathophysiological conditions in the eye that involve neurogenic inflammation and the release of CGRP.

Keywords: Calcitonin gene-related peptide (CGRP); vasodilatation; intraocular pressure; blood-aqueous barrier; cyclic AMP; methysergide; regional blood flow

Introduction

Neurogenic inflammation occurs in peripheral target tissues in response to sensory nerve stimulation (Jancso *et al.*, 1967) and is associated with inflammatory and protective states in many tissues, such as in airways, skin and eye (Lundberg & Saria, 1983; Caprioli *et al.*, 1985; Lundblad *et al.*, 1987). In the eye, neurogenic inflammation occurs in response to traumatic stimuli, such as laser therapy of the eye and ocular surgery (Caprioli *et al.*, 1985) and it consists of vasodilatation in the anterior uvea, breakdown of the blood-aqueous barrier, increase in the intraocular pressure (IOP) and miosis (Perkins, 1957).

In ocular neurogenic inflammation, a rapid vasodilatation in the anterior uvea disrupts the epithelial blood-aqueous barrier allowing plasma proteins to leak into the aqueous humour and at the same time it increases the intraocular pressure (Stjernschantz *et al.*, 1982; Unger *et al.*, 1985). Simultaneously the adenosine 3':5'-cyclic monophosphate (cyclic AMP) content in the aqueous humour is increased (Krootila, 1988). Neuropeptides localized in the sensory nerves are thought to be responsible for the changes in the neurogenic inflammation in the rabbit eye. Calcitonin gene-related peptide (CGRP) and substance P (SP) are both localized in the sensory nerves of the eye (Miller *et al.*, 1981; Tervo *et al.*, 1981; Terenghi *et al.*, 1985). They are released into the aqueous humour by antidromic electrical stimulation

of the ocular sensory nerves (Bill *et al.*, 1979; Wahlestedt *et al.*, 1986) and their effects mimic the effects of sensory nerve stimulation in the rabbit eye. CGRP causes vasodilatation in the anterior uvea, disruption of the blood-aqueous barrier, rise in the IOP and increase in the cyclic AMP content in the aqueous humour, while SP causes miosis (Stjernschantz *et al.*, 1982; Unger *et al.*, 1985; Krootila, 1988; Krootila *et al.*, 1988).

In a previous study we have demonstrated the inhibitory effect of methysergide on the IOP response in neurogenic inflammation in the rabbit eye (Krootila *et al.*, 1987). Because CGRP is thought to be responsible for the rise in IOP in neurogenic inflammation, the present study was carried out to investigate the effect of methysergide on CGRP-induced microvascular changes in the rabbit eye *in vivo* and on CGRP-induced vascular responses *in vitro*.

Methods

Animals and anaesthesia

Albino rabbits of either sex weighing 2.0–3.5 kg were used. For the experiments the animals were anaesthetized with intravenous urethane (1.5 g kg⁻¹, b.w., supplemented during the experiments). In order to prevent the production of prostaglandins all rabbits were pretreated with 50 mg of intravenous indomethacin 60 min before experiments.

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Experimental procedures

Methysergide (dissolved in physiological saline) was given intravenously at doses 1, 5 or 10 mg kg⁻¹, b.w. Human α -CGRP (0.5 or 2.0 μ g) was injected intracamerally with a Hamilton syringe, in a volume of 10 μ l, slowly, avoiding a rise in the IOP, 20–30 min after methysergide or saline. The control eyes received intracamerally saline (NaCl) correspondingly. The aqueous humour samples were taken 30 min after injection of CGRP or saline for the measurement of protein and cyclic AMP contents.

Recording of intraocular pressure (IOP) and blood pressure

For the continuous electromanometrical recording of blood pressure, the femoral artery was cannulated with a polyethylene tube and connected to a pressure transducer. The IOP of both eyes was continuously measured electromanometrically with special intracameral needles connected to pressure transducers and to a two-channel recorder. Following cannulations, the IOP was left to equilibrate for 30–60 min before further experiments.

Determination of regional blood flow

The regional blood flows were measured by use of radioactively labelled microspheres described in detail earlier (Krootila *et al.*, 1988; Oksala, 1988). After killing the animals the tissues were carefully dissected under the microscope, the ocular tissues were taken as a whole and the samples of the extra-ocular tissues were weighed after dissection. The results for ocular tissues are given as blood flow in mg min⁻¹/whole tissue and for extra-ocular tissues as blood flow in mg min⁻¹ g⁻¹ of tissue.

Measurement of protein and cyclic AMP contents in the aqueous humour

The protein content was measured according to the method of Lowry *et al.* (1951). For the measurement of cyclic AMP, the samples were mixed in 1:4 volume for 4 mM EDTA and measured with a ¹²⁵I-radioimmunoassay kit (Amersham RPA, 509, U.K.).

Contraction measurements on the isolated ophthalmic artery

New Zealand albino rabbits were killed by an overdose of sodium pentobarbitone and decapitated. The ophthalmic artery was isolated, cleaned of excess connective tissue and cut into 2 mm long pieces. The contraction experiments were performed isometrically at 37°C. The drugs were added to the incubation medium in desired concentrations. The composition of the incubation medium was (in mM): NaCl 138, KCl 5, MgCl₂ 1, CaCl₂ 2, glucose 10 and HEPES 10, pH 7.4.

Drugs

The following drugs were used: Urethane (Fluka Chemika, Switzerland), sodium pentobarbitone (Mebunat, Orion, Finland), methysergide hydrogenmaleate (Sandoz), human α -CGRP (Peninsula, Calif, U.S.A.), indomethacin (Confortid, Dumex, Denmark), noradrenaline HCl (Fluka Chemika).

Statistical analysis

Statistical comparison was made with Student's *t* test or matched-pair *t* test. Results are given as mean $\pm$ s.e.mean.

Results

Effect of methysergide on blood pressure, intraocular pressure and regional blood flow

Intravenous methysergide (1–10 mg kg⁻¹, b.w.) caused at 2–3 min a rapid and transient increase in the blood pressure, which recovered to control levels within 30 min (Table 1). In the IOP, a decrease occurred in 30 min after methysergide (i.v., 1–10 mg kg⁻¹, b.w., Table 2). In ocular tissues, decreases of 53–65% in regional blood flows were seen 20–30 min after 5 mg kg⁻¹ b.w., i.v. methysergide (Figure 1). In most other tissues studied, kidney, spleen, heart, brain and lung, vasoconstriction did not occur (Figure 2). Only in liver a modest decrease and in pancreas a modest increase in regional blood flow was seen (Figure 2).

Effect of methysergide on CGRP-induced inflammatory changes

Intracameral CGRP (2.0 μ g) caused typical inflammatory changes in the rabbit eye consisting of increase in the IOP, increased protein and cyclic AMP contents in the aqueous humour and vasodilatation. The doses 5 and 10 mg kg⁻¹ b.w. of methysergide (i.v.) inhibited the CGRP-induced changes in the IOP and protein and cyclic AMP contents in the aqueous humour, while the dose of 1 mg kg⁻¹ b.w. (i.v.) was ineffective (Figure 3, Table 3). Intracamerally administered 0.5 μ g CGRP increased the regional blood flow in the anterior uvea (iris and ciliary body) by 244.8 $\pm$ 80.2 mg min⁻¹/whole tissue. After methysergide (5 mg kg⁻¹, i.v.), the same dose of CGRP increased the regional blood flow only by 54.1 $\pm$ 23.3 mg min⁻¹/whole tissue (*P* < 0.025, Figure 4). This increase was at the same level as the increase after intracameral injection of corresponding volume of NaCl (33.8 $\pm$ 16.2 mg min⁻¹/whole tissue, Figure 4).

Contraction studies on isolated ophthalmic artery

Dose-response curves (DRC) for noradrenaline (NA) in the presence and absence of methysergide (10 nM) were performed (Figure 5). The contraction induced by NA 5 μ M in the absence of methysergide was 266 $\pm$ 15 mg and was regarded as 100% for further comparison. In the presence of methysergide, there was a shift of the DRC to the left and an increase in the maximal response to NA (Figure 5). Contraction

Table 1 Effect of methysergide on blood pressure (BP) in the rabbit

Dose (i.v.)	n	Before	BP (mmHg)	
			At 2–3 min	At 30 min
1 mg kg ⁻¹	5	101 $\pm$ 4	108 $\pm$ 5*	98 $\pm$ 5
5 mg kg ⁻¹	5	95 $\pm$ 2	110 $\pm$ 4**	96 $\pm$ 3
10 mg kg ⁻¹	13	102 $\pm$ 4	113 $\pm$ 5***	101 $\pm$ 4

Mean $\pm$ s.e.mean.

n = number of animals.

P* < 0.05; **P* < 0.001. Matched-pair *t* test.

Table 2 Effect of methysergide on intraocular pressure (IOP) in the rabbit

Dose (i.v.)	n	IOP (mmHg)	
		Before	At 30 min
1 mg kg ⁻¹	5	16.3 $\pm$ 0.6	15.0 $\pm$ 1.0*
5 mg kg ⁻¹	5	20.2 $\pm$ 1.5	16.5 $\pm$ 0.8*
10 mg kg ⁻¹	13	18.4 $\pm$ 0.6	14.7 $\pm$ 0.8***

Mean $\pm$ s.e.mean.

n = number of animals

P* < 0.05; **P* < 0.001. Matched-pair *t* test.

Figure 1 Effect of methysergide (5 mg kg^{-1} b.w., i.v.) pretreatment on regional blood flow in ocular tissues. $n = 9$. The statistical analysis was made between values before (open columns) and 20–30 min after methysergide (hatched columns) using matched-pair t test. $***P < 0.001$. Ret + Chor = retina and choroid; Scl + Corn = sclera and cornea; Iris + Cil body = iris and ciliary body. Mean values with s.e.mean shown by vertical bars.

Figure 2 Effect of methysergide (5 mg kg^{-1} b.w., i.v.) pretreatment on regional blood flow in extraocular tissues. $n = 9$. The statistical analysis was made between values before (open columns) and 20–30 min after methysergide (hatched columns) using matched-pair t test. $*P < 0.05$. Mean values with s.e.mean shown by vertical bars.

Figure 3 Effect of methysergide (1 mg kg^{-1} , ■, $n = 3$; 5 mg kg^{-1} , ▲, $n = 5$ or 10 mg kg^{-1} b.w., ●, $n = 6$) on the calcitonin gene-related peptide (CGRP)-induced ($2.0 \mu\text{g}$) increase in the intraocular pressure (IOP). The control animals (O, $n = 6$) received saline correspondingly before CGRP. $n =$ Number of eyes. $*P < 0.05$; $**P < 0.01$. Statistical comparison was made against the values at time 0 using matched-pair t test. Mean values with s.e.mean shown by vertical bars.

Table 3 Effect of methysergide on calcitonin gene-related peptide (CGRP)-induced ($2.0 \mu\text{g}$) increase in the protein and cyclic AMP content in the aqueous humour in the rabbit

Dose (i.v.)	n	Protein (mg ml ⁻¹)	Cyclic AMP (pmol ml ⁻¹)
Control (CGRP)	6	23.7 ± 2.3	243.2 ± 37.9
1 mg kg^{-1}	3	13.3 ± 6.2	147.0 ± 7.4
5 mg kg^{-1}	5	$0.6 \pm 0.04^{***}$	$34.6 \pm 3.1^{***}$
10 mg kg^{-1}	6	$0.6 \pm 0.1^{***}$	$65.3 \pm 14.7^{**}$

Mean $\pm$ s.e.mean.

$n =$ Number of animals.

$**P < 0.01$; $***P < 0.001$. Student's t test.

Figure 4 Effect of methysergide (5 mg kg^{-1} b.w.) on calcitonin gene-related peptide (CGRP)-induced ($0.5 \mu\text{g}$) vasodilatation in the anterior uvea. Number of animals is 7 in group receiving CGRP only and 9 in methysergide (methys) treated group. The results are given as blood flow in mg min^{-1} the whole tissue. $*P < 0.05$. Student's t test. Mean values with s.e.mean shown by vertical bars.

Figure 5 Dose-response curves for noradrenaline (NA) induced contractions in the rabbit isolated ophthalmic artery in the absence (O, $n = 8$) and presence (■, $n = 3$) of methysergide (10 nM). Contraction induced by $5 \mu\text{M}$ NA was regarded as 100%. Mean values with s.e.mean shown by vertical bars.

tions induced by $10 \mu\text{M}$ NA were $300 \pm 25 \text{ mg}$ and $710 \pm 150 \text{ mg}$ in the absence and presence of 10 nM methysergide, respectively. Methysergide (10 nM) alone did not cause any changes in the tone of isolated ophthalmic artery.

The inhibitory effect of CGRP on 5 μ M of NA-induced contraction in the absence and presence of methysergide (10 nM) and on 1 μ M of NA-induced contraction in the presence of methysergide (10 nM) were measured. Figure 6a shows an original tracing of an experiment where the inhibitory effect of increasing concentrations of CGRP on NA (5 μ M)-induced contraction was studied. NA (1 or 5 μ M) was added to the incubation solution and after the increase in tension was balanced, the NA was washed out. This was repeated until at least three contractions of the same size were obtained, whereafter the experiment was continued in the presence of increasing concentrations (0.1–3.0 nM) of CGRP. Figure 6b demonstrates the DRCs for increasing concentrations of CGRP on 5 μ M NA-induced contraction in the absence and presence of methysergide (10 nM) and on 1 μ M NA-induced contraction in the presence of methysergide (10 nM). There were no statistically significant differences in IC_{50} values for CGRP in these three DRCs.

Discussion

Methysergide is a nonselective 5-hydroxytryptamine (5-HT) antagonist at 5-HT₁ and 5-HT₂ receptors (Peroutka, 1988). It has been shown to contract the intracranial rabbit central ear artery *in vivo* (Apperley *et al.*, 1976), but not the intracranial artery in the cat *in vitro* (Hardebo *et al.*, 1978). Methysergide can also sensitize extracranial vessels to NA both *in vivo* and *in vitro* (de la Lande *et al.*, 1966; Schönbaum *et al.*, 1976). 5-HT receptors have been demonstrated in the rabbit eye (Mallorga & Sugrue, 1987). 5-HT was bound to the iris ciliary body membrane suspension and based on the ability of different antagonists to displace specific 5-HT binding, the binding sites were shown to be of the 5-HT₁ type. Methysergide had strong affinity for the 5-HT binding sites (Mallorga & Sugrue, 1987). In the present study, methysergide decreased the regional blood flow in ocular tissues *in vivo* but methysergide alone did not cause any contraction in the rabbit isolated ophthalmic artery *in vitro*. However, in the presence of methysergide, NA-induced contraction in the ophthalmic artery was potentiated *in vitro*. In this respect, the present findings are in agreement with the previous studies.

In tissues other than the eye, i.v. methysergide had very modest, if any, effects on regional blood flow. This demonstrates a tissue-specific mechanism of action of methysergide. It is possible that the eye is more sensitive to methysergide or there is perhaps local release of NA in the eye from the sympathetic nerves to interact with methysergide. Although in quiet, resting rabbits the sympathetic vasomotor tone to the eye is minimal, under urethane anaesthesia the small sympathetic tone might provide a source of NA for the eye (Bill & Nilsson, 1985).

The relatively intense vasoconstriction in the anterior uvea might explain the IOP lowering effect of methysergide. Although there was not a constant decrease in the regional blood flows in extra-ocular tissues, the transient increase in the blood pressure 2–3 min after the i.v. administration of methysergide might also reflect a transient systemic vasoconstriction.

In rabbit isolated ophthalmic artery, methysergide potentiated the contractile effect of NA. Methysergide has also 5-HT agonistic properties, as demonstrated earlier in other vascular tissues (Apperley *et al.*, 1980) and in rabbit ophthalmic artery (Zschauer *et al.*, 1991). It has also been shown that in the presence of 5-HT the contractile response to different agonists is enhanced (Seabrook & Nolan, 1983). Thus, potentiation of NA-induced contraction in the presence of methysergide in the rabbit ophthalmic artery might be due to 5-HT agonistic properties of methysergide. There were no differences in the IC_{50} -values for CGRP on the NA-induced contraction in the presence and absence of methysergide, indicating that methysergide has no direct effect on CGRP-induced vasodilatation *in vitro*. Thus, the

Figure 6 (a) Original tracing demonstrating the effect of increasing concentrations of calcitonin gene-related peptide (CGRP, 0.1–3.0 nM) on the noradrenaline (NA)-induced (5 μ M) contraction in the rabbit isolated ophthalmic artery. NA = addition of NA; W = wash out. (b) Dose-response curves (DRC) for CGRP on 5 μ M NA-induced contraction in the absence (●—●) and presence (■—■) of methysergide (10 nM) and on 1 μ M NA-induced contraction in the presence (Δ—Δ) of methysergide (10 nM). For each DRC for CGRP, the contraction before addition of CGRP was regarded as 100%. $n = 3$ in each. Mean with s.e.mean shown by vertical bars, except in (Δ), where the standard error bars (<15% in all points) have been omitted for clarity.

antagonistic effect of methysergide on CGRP-induced inflammatory changes in the rabbit eye is not direct. One of the mechanisms may be to enhance the effect of a vasoconstrictor (NA) to antagonize the vasodilator effect of CGRP.

It is also known that increased sympathetic vascular tone in the eye protects the eye from exogenous insult (Davson & Matchett, 1951) and that sympathectomized eyes show an enhanced responsiveness to noxious stimuli (Butler *et al.*, 1979).

CGRP is localized in the sensory nerves and is one of the most potent vasodilators yet discovered (Rosenfeld *et al.*, 1983; Brain *et al.*, 1985; Struthers *et al.*, 1986). In the eye, CGRP is localized in the trigeminal sensory nerves and CGRP-immunoreactive nerve fibres have been demonstrated in several species including man (Terenghi *et al.*, 1985; Stone & McGlinn, 1988; Uusitalo *et al.*, 1989). CGRP has been shown to be released into the aqueous humour in the rabbit by trigeminal nerve stimulation (Wahlestedt *et al.*, 1986) and CGRP is a vasodilator in the eye (Krootila *et al.*, 1988; Oksala, 1988). This CGRP-induced rapid vasodilatation in the anterior uvea is thought to cause disruption of the blood-aqueous barrier, subsequent leakage of plasma proteins into the aqueous humour and rise in the IOP in neurogenic inflammation (Stjernschantz *et al.*, 1982; Unger *et al.*, 1985). Thus, CGRP has been thought to act as a mediator in harmful ocular irritative responses (Unger *et al.*, 1985). In a previous study, we have demonstrated the inhibitory effect of methysergide on IOP response in neurogenic inflammation in the rabbit eye (Krootila *et al.*, 1987).

In the present study, the ocular effects of CGRP were inhibited by methysergide *in vivo*. Methysergide inhibited the CGRP-induced vasodilatation in the anterior uvea, increases in the protein and cyclic AMP contents in the aqueous

humour and rise in the IOP. In the rabbit eye, methysergide is a vasoconstrictor *in vivo* and this vasoconstriction seems to protect the eye from the vasodilator effect of CGRP. *In vivo*, methysergide decreased the CGRP-induced increase in regional blood flow in the anterior uvea, while *in vitro*, the magnitude of the CGRP-induced relaxation in the ophthalmic artery was the same in the absence and presence of methysergide. This suggests that *in vivo*, there might be also some other indirect mechanisms to explain how methysergide inhibits the CGRP-induced vasodilatation other than by enhancing the effect of a vasoconstrictor.

The present study addresses the pathophysiological effects of CGRP in the rabbit eye and our results provide the first

evidence that the ocular effects of CGRP can be inhibited with methysergide. The effect of methysergide has been shown to be tissue-specific and one of the mechanisms involved may be the potentiation of NA-induced vascular contraction. The present findings suggest that it is pharmacologically possible to limit some pathophysiological conditions in the eye that involve neurogenic inflammation and the release of CGRP.

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