Effects of Calcitonin Gene-Related Peptide in the Eye

A Study in Rabbits and Cats

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Calcitonin gene-related peptide (CGRP) has recently been demonstrated in sensory neurons of the eye. The purpose of the present study was to determine the effects of exogenous CGRP in the rabbit and cat eye. CGRP was injected intracamerally and the intraocular pressure was measured in cannulated eyes. The pupil diameter and the aqueous humor protein concentration were also measured. Indomethacin was used to prevent prostaglandin synthesis and tetrodotoxin (TTX) to block nerve conductance. In the rabbit eye, CGRP caused iridial hyperemia, a breakdown of the blood-aqueous barrier and increased intraocular pressure. These responses were dose-related. The increase in IOP as well as the breakdown of the blood-aqueous barrier could not be blocked with TTX or indomethacin. In cats CGRP caused a decrease in IOP and had only slight effect on the aqueous humor protein concentration. Neither in rabbits nor in cats had CGRP any detectable effect on the pupil size. Intracameral injection of 0.1 μg (7.4 × 10^{-11} moles) substance P together with 0.1 ng (2.6 × 10^{-11} moles) CGRP in rabbits caused maximal miosis but did not potentiate the intraocular effects of CGRP only. These results indicate that CGRP has marked vascular effects in the rabbit eye, causing a breakdown of the blood-aqueous barrier and increased IOP. The mechanism of this phenomenon does not involve prostaglandins neither nerve conduction, implying most likely a direct effect on the vascular smooth muscle. The mechanism of the decrease of IOP in cats remains unknown.

The eye typically reacts to noxious stimuli with miosis, hyperemia, breakdown of the blood-aqueous barrier and increased intraocular pressure (IOP). The mediators behind these responses are not known but generally, prostaglandins and sensory neurons are thought to be the most important factors. When the iris is traumatized directly, prostaglandins seem to be the key factor probably exerting most of their effects through an activation of sensory neurons. In nonperforated eyes the noxious impulse is thought to be propagated into the eye through an axon reflex mechanism. In these cases prostaglandins seems to be less important.

Calcitonin gene-related peptide (CGRP), a novel 37-amino acid polypeptide encoded within the calcitonin gene, has been shown to occur in the nervous system. CGRP has histochemically been localized to sensory neurons in the anterior segment of the eye. Substance P (SP) or a closely related tachykinin has been shown to be involved in the miotic component part of the acute irritative response of the rabbit eye but the vascular effects of SP in the anterior uvea of the rabbit have been reported to be much less marked. In the rabbit eye SP can be released from sensory nerves by trigeminal nerve stimulation and recently it was shown that CGRP can also be released into the aqueous humor of rabbits in a similar way. Thus, as a strong vasodilator, CGRP could very well be involved in the acute irritative response in the eye causing hyperemia, a breakdown of the blood-aqueous barrier and ocular hypertension. In previous studies CGRP has been demonstrated immunohistochemically and biochemically in the eye and the effects of exogenous CGRP have been demonstrated in the rabbit eye. The purpose of the present study was to analyze further the effects of exogenous CGRP in the rabbit eye and to investigate the effects of CGRP in the cat eye with special reference to the irritative response.

Materials and Methods

Albino rabbits weighing 2–3.5 kg and cats weighing 2–3 kg of either sex were used. The rabbits were anesthetized with 25% urethane (2 g/kg b.w.). The anesthesia of the cats was initiated with intramuscular injection of a combination of ketamine (20 mg/kg b.w.) and xylazine (0.5 mg/kg b.w.) and maintained with intravenous infusion of ketamine. In both rab-

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bits and cats the level of the anaesthesia was such that the corneal reflex was only weak or absent. All the animals received indomethacin 10 mg/kg b.w. intravenously 20 min before the cannulation of the eyes. The animals were kept warm by a thermostated heating pad. All the animals were tracheotomized to assure adequate ventilation and some of them were artificially ventilated. In all experiments the pH, P<sub>CO<sub>2</sub></sub> and P<sub>O<sub>2</sub></sub> of the arterial blood were checked routinely a few times during the course of the experiment (ABL 30; Radiometer, Copenhagen, Denmark). Artificial ventilation was adjusted to give normal or close to normal values for P<sub>CO<sub>2</sub></sub>, P<sub>O<sub>2</sub></sub> and pH. Both in rabbits and cats a femoral artery was cannulated and connected to a pressure transducer for blood pressure monitoring and in cats a femoral vein was also cannulated for administration of the anaesthetic.

The eyes were cannulated with 27 gauge needles connected to polyethylene tubings. Great care was taken not to touch the iris at any time during the experiment. The intraocular pressure and the blood pressure were measured with Statham P-50 pressure transducers (Gould, Inc., Oxnard, CA) connected to a Grass Model 79-D polygraph (Quincy, MA). Intracameral injections were carried out with Hamilton precision syringes. A volume of 5 μl was injected which caused a transient increase in IOP of 2-6 mm Hg lasting from 2 to 5 min.

An intravenous infusion of a 6% Dextran solution (Pharmacia, Uppsala, Sweden) diluted (1:1) with isotonic saline was used whenever needed to sustain the blood pressure. The infusion was started as soon as the mean arterial blood pressure fell below a level of about 70 mm Hg and was continued as long as necessary. The total amount of dextran solution did not exceed 50 ml.

The pupil diameter was measured with a transparent millimeter ruler under standard laboratory light. Iridial hyperemia was estimated macroscopically in albino animals. The hyperemia of the experimental eye was compared with that of the contralateral control eye, and was graded slight, medium or strong.

At the end of the experiment, paracentesis was performed with a 27 gauge needle and aqueous humor was stored at -50° until analyzed for protein concentration. Calcitonin gene-related peptide (α-CGRP (rat); Peninsula Laboratories, Belmont, CA), substance P (Peninsula), and tetrodotoxin (Sigma Chemical Company, St. Louis, MO) were dissolved in isotonic saline and stored at -50°C.

Experiments With Intracameral Injection of CGRP in Normal Eyes

In these experiments the eyes were cannulated with two needles, one for intraocular pressure measurement and one for administration of CGRP/vehicle. CGRP at 0.1–50.0 μg (2.6 × 10<sup>-11</sup>-1.3 × 10<sup>-8</sup> moles) was injected into the experimental eye after a stable baseline IOP was obtained (5–20 min after the second cannulation), while the other eye served as a control, receiving isotonic saline only.

Experiments With Intracameral Injection of CGRP in TTX Pretreated Eyes

In these experiments an additional needle was used for TTX administration. One microgram (3.1 × 10<sup>-9</sup> moles) of TTX was injected intracamerally bilaterally 10 min before the CGRP administration. Only rabbits were used. The effects of TTX denervation were estimated in six eyes by stimulating the preganglionic part of the sympathetic trunk electrically (10 Hz, 2 msec, 1 mA) before and after the TTX injection. Before the injection stimulation caused rapidly mydriasis, the pupil diameter reaching 10.1 ± 0.4 mm. The TTX injection itself caused a slight mydriasis, the pupil diameter being 8.7 ± 0.4 mm after TTX administration compared with 6.2 ± 0.1 mm prior to TTX injection. Ten minutes after the injection, sympathetic stimulation had no effect at all on the pupil size. All animals underwent in addition light stimulation in order to verify the blockade of cholinergic miosis. It was assumed that the unmyelinated sensory neurons are blocked in a similar way to the cholinergic and adrenergic neurons. The control eye received the vehicle only.

Experiments With Intracameral Injection of CGRP and SP

These experiments were performed only in rabbits. A mixture of 0.1 μg (7.4 × 10<sup>-11</sup> moles) of SP and 0.1 μg (2.6 × 10<sup>-11</sup> moles) of CGRP was injected intracamerally into four eyes. The control eye received the vehicle only.

The results are expressed as the arithmetical mean ± SEM, and were statistically evaluated with the student t-test for paired data by comparing the experimental eye with the control eye.

This research was carried out according to the guidelines of the ARVO Resolution on the Use of Animals in Research.

Results

Intracameral injection of CGRP into the rabbit eye caused hyperemia, a breakdown of the blood-aqueous barrier and increased intraocular pressure. CGRP at 0.1 μg (2.6 × 10<sup>-11</sup> moles) caused slight hyperemia in the iris; a dose of 1–5 μg (2.6 × 10<sup>-10</sup>-1.3 × 10<sup>-9</sup> moles) caused strong hyperemia. Intracameral injection of 0.1–5.0 μg (2.6 × 10<sup>-11</sup>-1.3...
Fig. 1. Effect of intracameral injection of 0.1 μg (A, n = 6), 0.5 μg (B, n = 6), 1–2 μg (C, n = 5) and 5 μg (D, n = 5) CGRP on the intraocular pressure in rabbits. Mean arterial blood pressure indicated by BP in the lower part of the respective picture. Experimental eyes indicated by solid line and control eyes by dotted line. Bars indicate SEM. * = P < 0.05, ** = P < 0.01 and *** = P < 0.001.

Fig. 2. Effect of intracameral injection of CGRP on the aqueous humor protein concentration in rabbits and cats. Bars indicate SEM. ** = P < 0.01.

X 10^-9 moles) CGRP caused a dose-dependent increase in IOP (Fig. 1) and in the aqueous humor protein concentration (Fig. 2). Intracameral injection of 10 ng (2.6 × 10^-12 moles) had no effect on the IOP or aqueous humor protein concentration. CGRP had no effect on the pupil size.

In cats intracameral injection of 5 μg (1.3 × 10^-9 moles) CGRP caused a decrease in IOP (Fig. 3). There were no macroscopic signs of ocular irritation in cats, but a tendency towards an increase (statistically insignificant) in the aqueous humor protein concentration was detected (Fig. 2). CGRP injected intracameral in as high a dose as 50 μg (1.3 × 10^-8 moles) in three cats caused only a slight increase in the aqueous humor protein concentration and did not elevate IOP.

Pretreatment of rabbit eyes with 1 μg (3.1 × 10^-9 moles) intracameral TTX, 10 min before injection of
Fig. 3. Effect of intracameral injection of 5 µg CGRP on the intraocular pressure in cats. Experimental eyes indicated by solid line and control eyes by dotted line. Bars indicate SEM. Mean arterial blood pressure indicated as in Figure 1, (n = 6). * = P < 0.05.

1 µg (2.6 × 10^{-10} moles) CGRP did not block the effects of CGRP on IOP or the aqueous humor protein concentration (Figs. 4, 5). TTX itself induced hyperemia and a slight but insignificant rise in IOP and the protein concentration of the aqueous humor.

Intracameral injection of a mixture of 0.1 µg (7.4 × 10^{-11} moles) SP and 0.1 µg (2.6 × 10^{-11} moles) CGRP induced maximal miosis but had no additional effect on IOP (Fig. 6), the hyperemia or the aqueous humor protein concentration (204 ± 58 mg/100 ml in the experimental eyes and 92 ± 6 mg/100 ml in the contralateral control eyes) as compared with the corresponding dose of CGRP only. If anything, the increase in IOP was smaller than with 0.1 µg CGRP only.

Discussion

Recent studies demonstrate that CGRP in the anterior uvea is confined to sensory neurons,10-13 is released by stimulation of the trigeminal nerve,19 and is a strong vasodilator in the eye.10,19 Thus, this potent polypeptide is a good candidate for one of the mediators of part of the irritative response of the eye, and possibly of antidromic vasodilatation in general.

It seems likely that the threshold dose of CGRP to induce a change in the parameters studied is in the range of 10–100 ng (2.6 × 10^{-12}–2.6 × 10^{-11} moles) when administered intracamerally in the rabbit. With a dose of 5 µg the upper limit was reached with a severe drop in blood pressure and contralateral effects. Less statistical significance was obtained in this group, eg, as compared with the 1 µg group (Fig. 2). Intracameral administration of as much as 100 µg (7.4 × 10^{-8} moles) of SP has been shown to have only little effect on the vasculature of the anterior uvea and the blood-aqueous barrier in urethane anaesthetized rabbits.18 Thus, compared with SP, CGRP seems to be of the order of magnitude 1000 times more potent as a vasodilator in the rabbit eye. This

mg/100 ml

** n=6

EXPERIMENTAL EYE

CONTROL EYE

Fig. 5. Effect of intracameral injection of 1 µg CGRP and isotonic NaCl on the aqueous humor protein concentration in rabbits which received an intracameral injection of TTX bilaterally 10 min prior to CGRP. Experimental eyes indicated by solid line and control eyes by dotted line. Bars indicate SEM. ** = P < 0.01.
estimation is in relatively good agreement with estimations of the vasodilatatory effects of SP and CGRP in the feline tooth pulp.21

The experiments performed in TTX-pretreated rabbits suggest that the effect of exogenous CGRP is not dependent on nerve conduction in the uvea. Of course, it cannot be excluded that CGRP may exert an effect through a prejunctional receptor directly coupled to substances stored in the nerve terminals. Strongly irritating agents, such as capsaicin and compound 48/80 seem to act through such a mechanism,22 whereas physiologic compounds such as prostaglandin E, histamine and bradykinin seem to require conduction in sensory nerves for function.6,17 Since both cholinergic and adrenergic neurons were blocked it is reasonable to assume that the unmyelinated sensory neurons were also blocked. Thus, it is probable that the effect of CGRP is a direct one on the vascular smooth muscle.

Although TTX in itself caused vasodilatation which could have rendered the eye more sensitive to edema and barrier breakdown, the fact remains that TTX did not induce any marked barrier breakdown but blocked nerve conduction. Control experiments with intracameral injection of isotonic saline only, in TTX-pretreated eyes, confirmed that the injection per se had no effect on the blood-aqueous barrier (Fig. 5).

The effect of intracameral injection of CGRP in cats was surprising, since the effect on the blood-aqueous barrier was very modest, and there was a decrease in IOP. Even when CGRP was injected in a dose as high as 50 μg (1.3 × 10⁻⁸ moles) intracamerally there was no increase in IOP and no significant barrier breakdown. The mechanism behind the decrease in IOP remains unknown, and merits further studies. Since the cat eye is considerably larger than the rabbit eye it can be assumed that intracameral injection of 5 μg CGRP in the cat eye corresponds to about 1 μg in the rabbit. Thus, it seems that there is a species difference in the response to CGRP between cats and rabbits. Since CGRP and SP coexist in sensory neurons of the anterior uvea,11,13 and SP has very little effect in the cat eye,23 it may simply be that the same is true for CGRP and that other systems are more important in sensory mechanisms in the cat eye.

Simultaneous injection of SP with CGRP did not appreciably increase the response to CGRP in rabbits; in fact, the increase in IOP and aqueous humor protein concentration was less than with CGRP only. This may be due to a decreased access of CGRP to the site of action due to instantaneous miosis. It should also be noted that there was a relatively strong decrease in systemic blood pressure of this group of animals after administration of the SP-CGRP mixture (Fig. 6). Thus, it seems that SP in the dose used does not potentiate the vascular effects of CGRP to any greater extent. Recently it was shown that low doses of CGRP potentiated the miotic effect of a low dose of SP as well as the weak vascular effects of SP.19 With the doses used in the present study it was not possible to show any potentiating effect of CGRP on the miotic effect of SP since the effect of SP was already maximal. The objective was rather to see if SP potentiates the vascular effects of CGRP. Thus, any substantial interaction between SP and CGRP in the anterior uvea of the rabbit does not seem to exist at the dose levels used.

The present study confirms that CGRP is a very potent vasodilator in the rabbit anterior uvea, causing breakdown of the blood-aqueous barrier. This effect does not seem to be mediated by neural elements or prostaglandins secondarily. In the cat, on the contrary, CGRP seems to have little inflammatory effect, if any, and a decrease was observed in IOP.

Key words: calcitonin gene-related peptide, intraocular pressure, aqueous humor protein concentration, blood-aqueous barrier, irritative response, nociception, rabbit, cat

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References