Intraocular and Cardiovascular Effects of Calcitonin Gene-Related Peptide (CGRP)-I and -II in the Rabbit

Kari Kroorila, Hannu Uusitalo, and Arto Palkama

Calcitonin gene-related peptide (CGRP) is a 37-amino acid neuropeptide localized in the eye in the sensory nerves. In this study, the physiological effects of the two naturally occurring forms of human CGRP, CGRP-I, and -II, which differ only in three amino acids, have been demonstrated in the rabbit eye and cardiovascular system. Intravenously administrated CGRP-I caused a biphasic increase in the intraocular pressure (IOP), disruption of the blood-aqueous barrier, and increase in the cyclic adenosine 3',5'-monophosphate (cAMP) content in the aqueous humor. CGRP-II caused a monophasic increase in the IOP and disruption of the blood-aqueous barrier, but no increase in the cAMP content occurred. CGRP-I and -II decreased the blood pressure in a similar dose-dependent manner. The effects of intracameraly administered CGRP-I and -II were very similar in the eye. An increase in the IOP, breakdown of the blood-aqueous barrier, and an increase in the cAMP content in the aqueous humor occurred. The differences in the biological responses between CGRP-I and -II in the rabbit eye might be a result of the different affinities of the CGRP forms to a single receptor. Alternatively, different subtypes of receptors for CGRP-I and -II may exist in the rabbit. Invest Ophthalmol Vis Sci 32:3084-3090, 1991

Materials and Methods

Animals and Anesthesia

Albino rabbits of both sexes weighing 2.0–3.7 kg were used. The animals were kept in a constant dark-light cycle (12 + 12 hours) and allowed food pellets and water ad libitum. The anesthesia was carried out using intravenous urethan (1.5 g/kg body weight), sup-
plemented during the experiments. The animals were tracheotomized and allowed to ventilate the room air freely. They received 50 mg of indomethacin (Confortid, Dumex, Denmark) intravenously 30-60 min before the experiments. All experiments were performed according to the ARVO resolution on use of animals in research.

Experimental Procedures

The human CGRP-I and -II were purchased from Peninsula Laboratories (Belmont, CA). CGRP was injected intravenously over a period of 3–5 min (CGRP-I: 0.1 μg/kg n = 3 (n = number of animals), 0.5 μg/kg n = 7, 2.5 μg/kg n = 6; CGRP-II: 0.1 μg/kg n = 3, 0.5 μg/kg n = 6, 2.5 μg/kg n = 6) or intracamerally (CGRP-I: 0.1 ng n = 3, 1 ng n = 3, 0.01 μg n = 4, 0.1 μg n = 4, 0.5 μg n = 7, 2.0 μg n = 5; CGRP-II: 0.1 ng n = 3, 1 ng n = 3, 0.01 μg n = 3, 0.1 μg n = 3, 0.5 μg n = 6, 2.0 μg n = 6). When injected intracamerally, the contralateral eye and both eyes of three control animals received saline intracamerally. In addition, six control animals received saline intravenously before taking the aqueous humor samples. The intracameral injections were performed slowly using a Hamilton syringe in a volume of 10 μl, avoiding a rise in the IOP. After 30 min the aqueous humor samples were taken, stored at -20°C until used for protein and cAMP analysis. The animals were killed with an overdose of anesthetics. The pupil diameter was measured with a millimeter ruler during the experiments.

Intraocular Pressure and Blood Pressure Measurements

The intraocular pressure (IOP) of both eyes was continuously measured electromanometrically using 27 gauge 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. The femoral artery of each animal was cannulated with a plastic catheter for continuous electromanometrical measurement of blood pressure during the experiment. The blood pressure values are given as mean arterial blood pressures. The heart rates of the animals were measured from the blood pressure recordings.

Aqueous Humor Analysis

The protein concentration in the aqueous humor was measured according to the method of Lowry et al. For the measurement of cAMP, the aqueous humor samples were immediately mixed in 4 mM ethylenediaminetetraacetic acid, stored at -20°C until assayed using a 125I-radioimmunoassay method (Amersham, RPA.509, U.K.).

Statistical Analysis

Statistical significance was determined using Student’s t-test between two means or a matched-pair t-test. The results are given as the arithmetical mean value ± standard error of the mean (mean ± SEM). After intravenous injections, the mean value of the changes in both eyes is regarded as the result from one animal. The changes in both eyes were usually equal.

Results

Intravenous Administration of CGRP

CGRP-I increased the IOP and decreased the blood pressure in a dose-dependent manner. No significant changes occurred at 0.1 μg/kg of CGRP-I (Fig. 1a). At 0.5 μg/kg, 3 out of 7 animals showed a biphasic IOP response curve, and at 2.5 μg/kg, 5 out of 6 animals showed a biphasic IOP response curve (Figs. 1b, 1c). The first peak occurred at the end of the intravenous (i.v.) injection, which lasted for 3–5 min, and the second peak occurred 15–20 min after the start of the injection. The blood pressure decreased rapidly in all animals and reached its lowest level at the end of the i.v. injection, after which the blood pressure started to recover (Figs. 1b, 1c).

CGRP-II at 0.5 and 2.5 μg/kg, but not at 0.1 μg/kg, increased the IOP and decreased the blood pressure (Fig. 2). However, the increase in IOP in response to the high dose of CGRP-II was not statistically significant. The most distinctive difference between CGRP-I and -II was that the IOP response had only one peak after CGRP-II. This peak value occurred at the end of the i.v. injection and no second peak appeared (Fig. 2). When significant changes occurred in IOP and blood pressure in response to CGRP-I and -II, the magnitudes were similar.

The highest doses (2.5 μg/kg) of both CGRP-I and -II increased the heart rate by 9 and 8%, respectively (both p < 0.05), while no changes occurred when the smaller doses were used. The maximal increase occurred at 3–5 min from the start of the injection.

The blood-aqueous barrier was disrupted after 0.5 and 2.5 μg/kg of CGRP-I and after 0.5 μg/kg of CGRP-II as judged from the increased protein content in the aqueous humor (Table 1). After CGRP-I, there were significant increases in the cAMP content in the aqueous humor. No increases were seen after CGRP-II (Table 1).

Finally, the pupil diameter did not change after either CGRP-I or -II.

Intracameral Administration of CGRP

Doses of CGRP-I and -II at 0.1 μg or less had no significant effects on IOP (Figs. 3a, 4a). At 0.5 and 2.0
Fig. 1. Effect of intravenously administered CGRP-I on IOP (□ — □) and mean arterial blood pressure (Δ — Δ). (A) 0.1 μg/kg. (B) 0.5 μg/kg. (C) 2.5 μg/kg. The peak values in IOP and the lowest values in blood pressure are indicated as black symbols. *P < 0.05. **P < 0.01. ***P < 0.001.

Fig. 2. Effect of intravenously administered CGRP-II on IOP (□ — □) and mean arterial blood pressure (Δ — Δ). (A) 0.1 μg/kg. (B) 0.5 μg/kg. (C) 2.5 μg/kg. The peak values in IOP and the lowest values in blood pressure are indicated as black symbols. *P < 0.05. **P < 0.01. ***P < 0.001.
Table 1. Effects of intravenous calcitonin gene-related peptide (CGRP) -I or -II on protein and cAMP contents in the aqueous humor

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of eyes</th>
<th>Protein content (mg/ml)</th>
<th>cAMP content (pmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>0.7 ± 0.1</td>
<td>28.5 ± 0.7</td>
</tr>
<tr>
<td>CGRP-I 0.1 µg/kg</td>
<td>3</td>
<td>1.0 ± 0.4</td>
<td>38.2 ± 4.8*</td>
</tr>
<tr>
<td>0.5 µg/kg</td>
<td>4</td>
<td>1.8 ± 0.4*</td>
<td>43.3 ± 3.8†</td>
</tr>
<tr>
<td>2.5 µg/kg</td>
<td>6</td>
<td>3.0 ± 0.3†</td>
<td>62.5 ± 7.3†</td>
</tr>
<tr>
<td>CGRP-II 0.1 µg/kg</td>
<td>3</td>
<td>0.7 ± 0.1</td>
<td>28.5 ± 0.6</td>
</tr>
<tr>
<td>0.5 µg/kg</td>
<td>6</td>
<td>1.7 ± 0.4*</td>
<td>47.6 ± 11.6</td>
</tr>
<tr>
<td>2.5 µg/kg</td>
<td>6</td>
<td>0.9 ± 0.1</td>
<td>31.6 ± 3.3</td>
</tr>
</tbody>
</table>

The control animals received saline intravenously. Values are mean ± SEM.
Statistical significances tested against the control group using t-test: *P < 0.05; †P < 0.001.

µg CGRP-I and -II, significant increases occurred in IOP (Figs. 3, 4). After the highest doses (2.0 µg) of CGRP-I or -II, increases were observed in the contralateral eyes.

No clear dose-dependent changes were observed in systemic blood pressure after intracameral CGRP.

In the aqueous humor, the protein content increased after the low doses of CGRP (0.01 µg and 0.1 µg, Table 2), while the IOP remained unchanged. No differences existed between CGRP-I and -II in the intensities of the disruption of the blood-aqueous barrier. Significant increases in the cAMP content in the aqueous humor were seen after CGRP-I and -II (Table 3). Here again, there were no differences in responses between CGRP-I and -II.

As after i.v. administration, no changes in the pupil diameter were seen after intracameral delivery of CGRP-I or -II.

Discussion

In the present study, intracameral administration of CGRP-I and -II had similar effects in the eye. After intravenous administration, the ocular effects were different, while the effects on the blood pressure were similar. Intravenously administered CGRP affects the eye from the endothelial side of the blood vessels. Intracamerally administered CGRP, on the other hand, affects the eye from the adventitial side of the blood vessels. Because endogenously released CGRP from the sensory nerves alters vascular permeability from the adventitial side of the capillary endothelium, the intracamerally delivered CGRP more closely resembles this release. Through both routes of administration, CGRP has been demonstrated to increase the blood flow in the anterior uvea of the rabbit eye.8,25

The most striking difference between the ocular effects of CGRP-I and CGRP-II after intravenous administration was that CGRP-I caused a biphasic increase in the IOP, while the IOP response after CGRP-II had only one peak. The first peak, which probably occurs as a result of the primary vasodila-
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Vol. 32

Fig. 4. Effect of intracameral administration of (A) 0.1 µg, (B) 0.5 µg, and (C) 2.0 µg of CGRP-II on IOP in the experimental and contralateral eyes. The contralateral eyes received intracamerally saline correspondingly. The peak values and the injection-induced peak (A) are indicated as black symbols. *P < 0.05, **P < 0.01, ***P < 0.001.

Table 2. Effects of intracameral calcitonin gene-related peptide (CGRP) -I or -II on protein content in the aqueous humor

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of eyes</th>
<th>Experimental eye</th>
<th>Contralateral eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>1.2 ± 0.1</td>
<td>—</td>
</tr>
<tr>
<td>CGRP-I 0.1 ng</td>
<td>3</td>
<td>0.8 ± 0.1*</td>
<td>—</td>
</tr>
<tr>
<td>1 ng</td>
<td>3</td>
<td>2.4 ± 0.9</td>
<td>—</td>
</tr>
<tr>
<td>0.01 µg</td>
<td>3</td>
<td>2.3 ± 0.5*</td>
<td>—</td>
</tr>
<tr>
<td>0.1 µg</td>
<td>4</td>
<td>4.6 ± 1.3†</td>
<td>—</td>
</tr>
<tr>
<td>0.5 µg</td>
<td>5</td>
<td>13.6 ± 2.3‡</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>2.0 µg</td>
<td>5</td>
<td>18.7 ± 3.3‡</td>
<td>1.3 ± 0.6 (N = 4)</td>
</tr>
<tr>
<td>CGRP-II 0.1 ng</td>
<td>3</td>
<td>0.9 ± 0.1</td>
<td>—</td>
</tr>
<tr>
<td>1 ng</td>
<td>3</td>
<td>2.2 ± 1.3</td>
<td>—</td>
</tr>
<tr>
<td>0.01 µg</td>
<td>3</td>
<td>3.2 ± 1.5</td>
<td>—</td>
</tr>
<tr>
<td>0.1 µg</td>
<td>3</td>
<td>5.0 ± 2.5*</td>
<td>—</td>
</tr>
<tr>
<td>0.5 µg</td>
<td>6</td>
<td>10.0 ± 2.5‡</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>2.0 µg</td>
<td>6</td>
<td>14.2 ± 2.5‡</td>
<td>4.8 ± 1.1†</td>
</tr>
</tbody>
</table>

The control animals and the contralateral eyes received saline intracamerally. Values are mean ± SEM. Statistical significances are tested against the control group using t-test: *P < 0.05; †P < 0.01; ‡P < 0.001.

dose of CGRP is increased (and the blood pressure is decreased). Furthermore, after intravenous administration, cAMP content in the aqueous humor was increased only after CGRP-I. Whether the increase in the cAMP content is related to the second peak in the IOP response has not been determined.

Recently, Anderson and Bill suggested that the breakdown of the blood-aqueous barrier occurs in two parts. 26 First, the plasma proteins extravasate...
from the capillaries to the stroma of the ciliary body but are still retained by the epithelial connections between the nonpigmented epithelial cells. At the second phase, the epithelial barrier disrupts and the extravasated plasma proteins are allowed to leak into the aqueous humor. This two-step breakdown of the blood-aqueous barrier could explain the biphasic increase in the IOP. However, after 0.5 μg/kg of intravenous CGRP-II the blood-aqueous barrier was also disrupted, and after intracamer al administration both forms of CGRP had similar effects on the blood-aqueous barrier. Also possible is that a second mediator causes the second peak in the IOP response after intravenous administration of CGRP-I.27-29 Although indomethacin decreases the effects of i.v. CGRP on the blood-aqueous barrier in the rabbit eye,26 this second mediator is unlikely to be a metabolite of arachidononic acid in the cyclo-oxygenase pathway because in the present study the rabbits were pretreated with indomethacin. Also possible is that after intravenous administration, the two forms of CGRP have different actions on nonocular sites, eliciting dissimilar indirect ocular effects, either totally or only partially (second peak). The similarity of the ocular effects after intracameral CGRP-I and -II suggests that ocular active compound is released from nonocular sites after intravenous administration of CGRP-I.

The second peak in the IOP response after intravenous CGRP-I mimics the contralateral response in the IOP observed after sensory nerve stimulation and after intracameral CGRP.7,30 It has been suggested that CGRP released into the systemic circulation from the eye causes this contralateral response in IOP.28 It is also possible that CGRP-I released from the eye in systemic circulation releases some ocular active mediator.

CGRP has a direct positive inotropic and chronotropic effect on the isolated heart from rats and guinea pigs, but not in rabbits.9-11 In humans, CGRP-I and -II have a positive inotropic effect on isolated auricles and a positive inotropic and chronotropic effect in vivo.20 In this study CGRP-I and -II caused a minor increase in the heart rate only after the highest dose (2.5 μg/kg), although significant decreases in blood pressure occurred after the lower doses. The evidence from the earlier studies32-33 and from this study suggest that this increase in the heart rate is reflexive because of the hypotension, not a direct effect on the heart.

The human CGRP-I and -II differ only in three amino acid residues, at positions 3, 22, and 25. Recent studies have shown that different CGRP epitopes have been studied to determine the active sites on the molecule. The epitope 8-37 of human CGRP antagonized the binding of the rat CGRP to rat liver plasma membrane.34 The same epitope was demonstrated to be an antagonist for human CGRP-I in guinea pig atrial and ileal preparations and in the central nervous system but not in other biological assays,35 leading to a suggestion that there are at least two classes of CGRP receptors.36 The differences between the biological responses of these two peptides may reflect the existence of different receptor subtypes for CGRP or different affinity of the CGRP molecules to the receptor in the rabbit. Based on the present study, changes in amino acids at the positions 3, 22, and 25 of CGRP alter its effects, directly or indirectly, on IOP, blood-aqueous barrier, and cAMP production by the iris-ciliary body in the rabbit.

**Key words:** calcitonin gene-related peptide, intraocular pressure, blood-aqueous barrier, cAMP, blood pressure

### Acknowledgments

The authors wish to thank Mrs. Aune Alsi for excellent technical assistance, Miss Majja-Leena Johansson for secretarial help and Prof. Britt Bromberg for valuable comments on the manuscript.

### References


