Intraocular effects of substance P in the rabbit

Johan Stjernschantz, Marvin Sears, and Lena Stjernschantz

The intraocular effects of substance P (SP) were studied in rabbits by measuring the pupil diameter, intraocular pressure (IOP), and aqueous humor protein concentration. Most of the animals were pretreated with indomethacin to avoid any interaction with prostaglandins. Intracameral injection of 1 to 150 ng of SP caused strong and persistent miosis without appreciably affecting the aqueous humor protein concentration or IOP. Intracameral injection of 0.8 to 11 µg of SP also induced an increase in IOP (7 to 8 mm Hg) without any apparent concomitant disruption in the blood-aqueous barrier. Outflow facility of aqueous humor decreased by a mean value of 50% after intracameral injection of 0.8 to 1.5 µg of SP. Since the increase in IOP could be prevented by iridectomy, it was probably caused by a pupillary block from the intense miosis induced by SP. No disruption in the blood-aqueous barrier could be detected after intra-arterial infusion of 100 µg of SP or intravitreal injection of 100 ng of SP, indicating that the ciliary epithelium was practically insensitive to exogenous SP. Topical as well as subconjunctival administration of up to 1 mg of SP did not cause any irritative response in the eye. The results show that with concentrations of SP causing intense miosis, the eye does not exhibit visible hyperemia and disruption of the blood-aqueous barrier. This finding is consistent with the hypothesis that after certain irritative stimuli, miosis is mediated by a pathway separate from the hyperemia and disruption of the blood-aqueous barrier.

Key words: substance P, rabbit, pupil size, aqueous humor protein concentration, ocular irritative response, blood-aqueous barrier, intraocular pressure, outflow facility, nociceptive stimuli

The response of the eye to an irritative stimulus undoubtedly depends on one or more chemical mediators. It has been shown that the response to a mechanical irritant such as paracentesis is mediated by prostaglandins, and that a neural pathway is probably unimportant. On the other hand, the acute response to a painful stimulus such as nitrogen mustard or formaldehyde is apparently not dependent on the synthesis or release of prostaglandins but instead is mediated by nerves. Recently it was shown that stimulation of the trigeminal nerve in rabbits causes an increase in substance P (SP)-like immunoreactivity of the aqueous humor and that intracameral infusion of SP simulated the irritative response during stimulation, comprising miosis, a breakdown in the blood-aqueous barrier, and elevated intraocular pressure (IOP). SP has immunohistochemically been localized to primary sensory neurons, and it was proposed that SP may be the agent involved in the nerve-mediated irritative response of the eye. It has also recently been reported that the

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53
Table I. Effect of intracameral injection of SP on the pupil diameter

<table>
<thead>
<tr>
<th>Amount injected (pg)</th>
<th>n</th>
<th>SP eye (mm)</th>
<th>Control eye (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>1</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>120</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>500</td>
<td>1</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>500</td>
<td>1</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>1-3.5</td>
<td>4</td>
<td>1.2 ± 0.3*</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>85-150</td>
<td>5</td>
<td>≤1</td>
<td>5.2 ± 0.2</td>
</tr>
</tbody>
</table>

*Statistical significance at the p < 0.001 level between the experimental and control eyes

iris-ciliary body preparation of the rabbit contains substantial amount of SP, most of which can be depleted by sensory denervation. The purpose of the present study was to analyze in more detail the intraocular effects of SP as they relate to the ocular response to nociceptive stimuli.

Materials and methods

Male albino rabbits, weighing 2 to 3 kg, were used. Most of the animals were anesthetized with urethane (1.5 to 2 gm/kg b.w.), but part were kept unanesthetized to check the effect of anesthesia on the parameters studied. Before cannulation of the eyes and paracentesis, and mostly before measuring the eye pressure with a pneumotonometer in unanesthetized animals, 2 drops of 0.5% proparacaine hydrochloride (Alcaine) were applied on the cornea. Most of the animals were tracheotomized, and in all the experiments with cannulation eyes a femoral artery was cannulated for blood pressure measurement. Most of the animals also received 25 to 50 mg of indomethacin intravenously 5 to 90 min before administration of SP.

Administration of SP. Synthetic SP (Sigma Chemical Co.) was dissolved in isotonic saline and administered in one of the following ways. In all experiments the same volume of an isotonic saline solution was administered to the control eye in the same way as SP was administered to the experimental eye.

Topical instillation. Two drops (100 μl) of a solution containing the appropriate amount of SP were applied on the cornea. The solution was not washed away.

Subconjunctival injection. A solution of 200 μl containing the appropriate amount of SP was injected subconjunctivally at the upper and lower conjunctival sac with a 27-gauge needle.

Intravitreal injection. A solution of 10 μl containing the appropriate amount of SP was injected intravitreally with Hamilton precision syringes through a 27-gauge needle inserted about 3 mm posterior to the limbus. After the experiment the eyes were opened to check that no bleeding had occurred and that the lens was intact.

Intracameral injection. A solution of 5 μl containing the appropriate amount of SP was injected with a Hamilton precision syringe into the anterior chamber through a cannula used for IOP registration. The injection of a 5 μl volume raised the IOP 7.4 ± 0.3 mm Hg (n = 54), but within 2 to 5 min the pressure had returned to about the preinfusion level. In all experiments IOP of the experimental eye was always compared with that of the control eye.

Intra-arterial infusion. A branch of the common carotid artery, mostly a thyroidal artery, was cannulated in a distal-proximal direction so that the tip of the tubing protruded to a distance of about 2 to 3 mm from the common carotid artery. A solution of 0.5 to 1 ml containing the appropriate amount of SP was then infused into the common carotid artery during a 10 to 30 sec period.

Cannulation of the eyes, paracentesis, and measurement of IOP. The eyes were cannulated with a needle gun having special needles for IOP measurement. Care was taken not to scratch the iris, and animals with iridal trauma were discarded. In the experiments with measurement of outflow facility, two needles were fired into each eye. Paracentesis was performed at the end of the experiment with a 22-gauge needle attached to a polyethylene tubing. In the eyes cannulated, IOP was measured with pressure transducers connected to a four-channel recorder. In the rest of the rabbits the eye pressure was measured with an Alcon pneumotonometer, calibrated for pressure measurements in rabbits.

Determination of outflow facility. The outflow facility of aqueous humor was measured with a two-rate continuous-infusion method, essentially as described by Sears.9 About 30 min after injection of SP when IOP had equilibrated to a new level (preinfusion pressure), a bilateral infusion of an electrolyte solution10 with a rate of 0.99 μl/min was started and continued for 15 to 20 min. IOP usually reached a new steady-state level after 10 to 15 min of infusion, and a pressure level over a 5 min period was then obtained. After this the rate of infusion was increased to 1.97 μl/min, and a new steady-state level of IOP could be obtained within 15 to 20 min as before. The infusion was then closed, IOP was left to equilibrate for about...
Fig. 1. Protein concentration of the aqueous humor 30 to 60 min after intracameral injection of SP. The amounts of SP injected are indicated below the columns. The group which received 7.5 to 11 μg of SP was not pretreated with indomethacin. Empty columns represent control eyes, and shaded columns SP eyes. Asterisk, Statistical significance at the p < 0.001 level. Mean ± S.E.M.

Table II. Effect of intracameral injection of SP on IOP

<table>
<thead>
<tr>
<th>Injected amount</th>
<th>SP eye (mm Hg)</th>
<th>Control eye (mm Hg)</th>
<th>Maximal pressure after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5-3.5 ng</td>
<td>6</td>
<td>17.7 ± 1.8</td>
<td>18.6 ± 2.1</td>
</tr>
<tr>
<td>85-150 ng</td>
<td>5</td>
<td>17.5 ± 0.8</td>
<td>19.7 ± 1.2*</td>
</tr>
<tr>
<td>0.8-1.2 μg</td>
<td>8</td>
<td>18.5 ± 1.5</td>
<td>24.8 ± 1.9†</td>
</tr>
<tr>
<td>7.5-11 μg</td>
<td>9</td>
<td>17.6 ± 1.5</td>
<td>23.5 ± 3.2†</td>
</tr>
</tbody>
</table>

The maximal pressure level was obtained about 30 to 40 min after injection. SP eyes were compared with the control eyes.

*Statistical significance at the p < 0.02 level.
†Statistical significance at the p < 0.01 level.

20 to 30 min, and the postinfusion pressure was obtained. Outflow facility was calculated according to the formula: C = F/ΔP, where F = the rate of infusion, and ΔP = the change in the pressure induced by infusion. The value of outflow facility during the first infusion was obtained by using the preinfusion pressure in calculating ΔP. The value of outflow facility during the second infusion was obtained with the use of the postinfusion pressure in calculating ΔP.

**Determination of aqueous humor protein concentration.** The protein concentration of the aqueous humor was determined with the Coomassie brilliant blue protein dye-binding method. The aqueous humor samples were stored in −20°C and were assayed for protein concentration within 3 weeks after the experiment.

**Measurement of pupil size.** The pupil diameter was measured with a millimeter ruler at a distance of about 1 cm from the eye under ordinary laboratory light, kept the same during all the experiments.

**Iridectomy.** In eight animals a peripheral iridectomy was performed 4 to 6 weeks before the experiment. One half of the animals were unilaterally iridectomized, and one half were bilaterally iridectomized. The triangular iridectomy was usually 2 to 3 mm long and 1 to 2 mm broad at the base. The eyes were examined before the experiment for signs of ocular inflammation, and only those animals without inflammatory signs were used. One half of the animals received indomethacin before the experiment.

The results are presented as the arithmetical mean value ± S.E.M. (n) and were statistically evaluated with Student's t test of paired data.
Table III. Outflow facility of aqueous humor after intracameral injection of 0.8 to 1.5 μg of SP

<table>
<thead>
<tr>
<th>Eye</th>
<th>Infusion 1 (0.99 μl/min) (μl/min/mm Hg)</th>
<th>Infusion 2 (1.97 μl/min) (μl/min/mm Hg)</th>
<th>Mean value (μl/min/mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP</td>
<td>0.17 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>Control</td>
<td>0.34 ± 0.03*</td>
<td>0.29 ± 0.05*</td>
<td>0.32 ± 0.04*</td>
</tr>
</tbody>
</table>

*Statistical significance at the p < 0.01 level. (n = 7).

Results

Topical application of 0.1 to 1 mg of SP (n = 5) had no or only a minute, transient decreasing effect on the pupil diameter and caused no visible conjunctival hyperemia. There was no increase in aqueous humor protein concentration or IOP. Subconjunctival injection of up to 1 mg of SP (n = 3) had no clear effect on the parameters studied.

Intracameral injection of 60 to 120 pg of SP (n = 3) had no effect on the pupil diameter; however, 500 pg in two experiments clearly decreased the pupil diameter, and 1 to 3.5 ng of SP (n = 4) caused a marked and persistent miosis within 5 min after injection (Table I). There was no increase in aqueous humor protein concentration or IOP (Fig. 1 and Table II). Intracameral injection of 85 to 150 ng of SP caused intense miosis within 2 to 3 min after injection (Table I), but some increase in IOP (p < 0.02) as compared with the contralateral eye (Table II). Intracameral injection of 0.8 to 1.2 μg of SP (animals pretreated with indomethacin) as well as 7.5 to 11 μg of SP (animals not pretreated with indomethacin) rapidly caused intense miosis, some increase in the aqueous humor protein concentration (Fig. 1), and elevated IOP, 6.6 ± 1.3 mm Hg and 8.1 ± 2.3 mm Hg, respectively (p < 0.01) (Table II). The increase in IOP usually started 6 to 15 min after injection of SP, and the maximal pressure level was reached after 10 to 20 min and was usually stable for a period of about 30 to 40 min.

There was no clear correlation between the increase in aqueous humor protein concentration and the increase in IOP (Fig. 2). Table III shows the effect of intracameral injection of 0.8 to 1.5 μg of SP on outflow facility of aqueous humor calculated from the pressure rise during infusion with two different rates. Outflow facility decreased with a mean value of 50% after injection of SP as compared with the contralateral eye (p < 0.01).

The IOP in previously iridectomized rabbits did not increase upon intracameral injection of 6 to 11 μg of SP (Table IV). The aqueous humor protein concentration of the iridectomized experimental eyes was slightly elevated over that of the contralateral control eyes, 186 ± 43 mg/dl (n = 8) and 83 ± 14 (n = 8), respectively (p < 0.05). The protein concentration of the aqueous humor in the iridectomized control eyes was 100 ± 23 mg/dl (n = 4). The corresponding figure for the unoperated control eyes of the same group was 65 ± 14 mg/dl (n = 4). There was no significant difference in the aqueous humor protein concentration between animals pretreated and those not pretreated with indomethacin.

Intravitreal injection of 100 ng of SP (n = 4) caused a moderate but definite decrease in pupil diameter but no other changes. The response in pupil size usually appeared 2 to 2.5 hr after injection. Infusion of 100 μg of SP into the common carotid artery caused a persistent ipsilateral miosis but no significant change in IOP or aqueous humor protein concentration on the side of infusion (Table V). There was no visible hyperemia of the iris or the conjunctiva. The arterial blood pressure rapidly dropped during infusion, with a mean value of 46 ± 3 mm Hg, but usually recovered to the normal level within 10 to 15 min.

The mean damped pressure in the femoral artery of anesthetized rabbits was 86 ± 2 mm Hg at the beginning of the experiment and 80 ± 2 at the end.
Table IV. Maximal change in IOP at equilibrium after intracameral injection of 6 to 11 μg of SP in iridectomized and normal eyes

<table>
<thead>
<tr>
<th>Iridectomized eyes</th>
<th>Normal eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in IOP</td>
<td></td>
</tr>
<tr>
<td>n/mm Hg</td>
<td>n/mm Hg</td>
</tr>
<tr>
<td>8/−1.3 ± 0.8</td>
<td>9/8.1 ± 2.3*</td>
</tr>
</tbody>
</table>

The group of normal eyes is identical to the group with 7.5-11 μg of SP injection in Table II. *Statistical significance at the p < 0.01 level.

Table V. Effect of intra-arterial infusion of 100 μg of SP on pupil size, aqueous humor protein concentration, and IOP at equilibrium

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>SP eye</th>
<th>Control eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupil diameter (mm): Before infusion</td>
<td>5.5 ± 0.4</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>After infusion</td>
<td>1.5 ± 0.4*</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>Intraocular pressure (mm Hg): Before infusion</td>
<td>17.8 ± 0.9</td>
<td>17.4 ± 0.8</td>
</tr>
<tr>
<td>After infusion</td>
<td>17.6 ± 1.2</td>
<td>16.0 ± 0.6</td>
</tr>
<tr>
<td>Aqueous protein concentration 30-40 min after infusion (mg/100 ml)</td>
<td>67 ± 17</td>
<td>37 ± 4</td>
</tr>
</tbody>
</table>

*Statistical significance at the p < 0.001 level. (n = 8)

Discussion

In 1960 Sears suggested that miosis and alteration of IOP were manifestations of two separate pathways to painful stimuli. Recently Maul and Sears again presented evidence suggesting that there were separate pathways for miosis and hyperemia. In the present study it has been clearly shown that SP can indeed cause a miosis in doses that do not cause hyperemia, barrier breakdown, or pressure rise, and this finding strengthens the idea that there may be a separate path for miosis and a second or other paths for the other three components.

In a previous study it was shown that part of the increase in IOP and breakdown of the blood-aqueous barrier after intracameral injection of SP was related to release of prostaglandins and could be blocked by pretreatment with indomethacin. In the present work most of the animals were therefore pretreated with indomethacin to avoid any possible effect of prostaglandin synthesis and release. The group with intracameral injection of 7.5 to 11 μg of SP not pretreated with indomethacin, however, showed that there was probably no release of prostaglandins upon intracameral administration of SP. The difference between these experiments and those described earlier may very well be due to different anesthetics. In earlier experiments, pentobarbital was used as an anesthetic. Pentobarbital has been shown to increase the blood flow of the anterior uvea strongly, and it is conceivable that this may render the barrier more sensitive to breakdown, possibly on the basis of a release of prostaglandins.

Topical application of SP in amounts up to 1 mg had no effect or only a weak one on the pupil diameter and caused no alterations in the aqueous humor protein concentration or

Fig. 2. Correlation between the change (difference between SP and control eye) in aqueous protein concentration and the change in IOP after intracameral injection of 0.8 to 11 μg of SP. Each point represents one separate animal. The points are scattered randomly, indicating that the change in IOP was not linked to a change in barrier permeability.
Fig. 3. Immediate effect of intra-arterial infusion of SP on IOP and blood pressure. Note the transient increase in IOP of the SP (ipsilateral) eye, indicating vasodilation.

IOP. This indicates that although SP is a putative sensory neurotransmitter, the peripheral pain receptor–stimulating property of SP is weak, an observation in agreement with that made by Lembeck et al. Since sometimes high doses of SP given topically caused a little decrease in pupil diameter, usually 10 to 15 min after instillation, it is probable that traces of SP may diffuse through the cornea. The fact that subconjunctival injection of SP was totally ineffective was somewhat surprising. This may indicate that SP is quickly degraded by the conjunctival tissue. It is possible that most of the SP intravitreally injected was picked up by the retina, a tissue known to contain considerable amounts of SP. The amounts that reached the posterior chamber, as evident from the decrease in pupil size, did not cause any breakdown of the ciliary epithelium.

The smallest amount of SP intracameraly injected to induce a definite decrease in pupil diameter was 500 pg. Assuming that the volume of the anterior chamber is 250 μl, it can be calculated that the aqueous humor concentration of SP (MW about 1500) must have been of the order of 1 to 2 × 10⁻⁹ M. If SP were adsorbed to the plastic surface of the tubings, the actual concentration may have been lower. Intracameral injection of a 200-fold larger amount of SP caused intense miosis and a tendency toward increased IOP but no change in aqueous humor protein concentration. Only with amounts of 0.8 to 11 μg, a small and variable increase in aqueous humor protein concentration was observed, possibly reflecting a slight disruption in the blood-aqueous barrier. There was, however, no correlation between the increase in IOP and the increase in aqueous humor protein concentration (Fig. 2), and therefore the possibility that the increase in IOP would have been secondary to a slight breakdown in the blood-aqueous barrier is unlikely.

Most of the increase in IOP undoubtedly was due to a decrease in the outflow facility of aqueous humor. Since the increase in IOP could be prevented by iridectomy, it is evident that the mechanism was pupillary block with secondary iridocorneal obstruction of the chamber angle. Intracameral injection of small amounts of SP could induce miosis without increasing IOP. Small differences, impossible to measure macroscopically, between a maximally and submaximally constricted pupil could very well decide whether or not pupillary block occurs. Interestingly, Al-Ghadyan et al. presented evidence that part of the increase in IOP seen after paracentesis in rabbits is secondary to a pupillary block. The potential for the same mechanism exists in any eye with intense miosis.

Infusion of 100 μg of SP into the common carotid artery resulted in an immediate ipsilateral constriction of the pupil and a transient increase in IOP simultaneously with a decrease in the contralateral IOP and the arterial blood pressure (Fig. 3). The reasonable explanation for this pressure behavior is no doubt marked vasodilation in the ipsilateral eye. It is therefore evident that SP must have reached the anterior uvea, although it is not clear what the actual amounts of SP reaching...
the eye were. Since the capillaries of the ciliary processes are very permeable even to large molecules like plasma proteins, part of the bolus was likely to leak into the stroma of the ciliary processes. It was very surprising then that no leakage of proteins into the aqueous humor occurred in the ipsilateral eye. In a recent study it was shown that no leakage of proteins into the stroma during electrical stimulation of the trigeminal nerve there is a strong leakage of albumin out of the ciliary capillaries and a breakdown of the epithelial barrier, in spite of the very low blood pressures. The question therefore arises whether SP is involved at all in the breakdown of the blood-aqueous barrier following nociceptive stimuli, and the present study suggests that the disruption of the blood-aqueous barrier is rather dependent upon a release of some other agent from sensory neurons.

A recent publication indicated that rabbits pretreated with capsaicin do not develop the initial increase in IOP after topical application of nitrogen mustard. Capsaicin has been shown to deplete sensory nerves of SP, and it was suggested that SP or a related peptide causes the rise in IOP after nitrogen mustard. The work reported herein shows that in concentrations that produce intense miosis, no hyperemia nor disruption of the barrier occurs in the eye. This observation means that capsaicin depletes not only SP but probably other sensory nerve components as well and that these others may well be responsible for the vascular component of the ocular irritative response.

The results of the present study suggest that the miosis following nociceptive stimuli is mediated by SP, and that part of the increase in IOP may be secondary to a pupillary block induced by the intense miosis. Exogenous SP does not cause much disruption of the blood-aqueous barrier, which indicates that some other agent is likely to cause this response. These findings are consistent with the hypothesis that miosis is mediated through a separate pathway and/or mechanism. Experiments with exogenous SP do not, however, exclude the possibility that endogenously released SP may have a different action on the blood-aqueous barrier and IOP. Investigations to evaluate this point are in progress.

REFERENCES


