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Substance P in proliferative vitreoretinopathy: the significance of aqueous humor levels for evolution of the disease

Abstract • Purpose: We detected aqueous humor levels of substance P in patients with various grades of proliferative vitreoretinopathy and with uncomplicated rhegmatogenous retinal detachment. To evaluate the significance of the concentration of substance P at the time of surgery for retinal detachment for subsequent development of proliferative vitreoretinopathy, the latter patients also underwent fundoscopic control examination. • Methods: Using a highly specific and sensitive radioimmunoassay, the content of substance P in fresh samples of aqueous humor obtained by paracentesis was determined both in cataract controls and in patients with uncomplicated rhegmatogenous retinal detachment and with various grades of proliferative vitreoretinopathy. Retinal detachment patients underwent fundoscopic control examination 6 months after surgical reattachment. • Results: The mean concentration of substance P in cataract controls was 40.3 (+22.4) fmol/mg protein, in the retinal detachment group 61.9 (+13.9) fmol/mg protein and in proliferative vitreoretinopathy 335.2 (+24.8) fmol/mg protein, but no correlation between levels of the peptide and various grades of the disease was observed. Already at surgery for retinal detachment three in four patients who developed proliferative vitreoretinopathy presented with levels of substance P in the range of the disease. • Conclusion: The concentration of substance P in aqueous humor is significantly high in patients with proliferative vitreoretinopathy in whom surgery is indicated. Furthermore, elevation of the peptide in retinal detachment that originates most obviously from a neurogenic mechanism may indicate initiation of processes associated with proliferative vitreoretinopathy, thus representing an indicator of significant risk for evolution of the disease at a very early time.

Introduction

Substance P (SP) belongs to a family of bioactive peptides, the tachykinins, which are widely distributed throughout the central and peripheral nervous system and act as neurotransmitters and/or neuromodulators. In the retina, SP immunoreactivity (IR) has been found within specialized amacrine and ganglion cells in various species, and their dendrites ramify predominantly in laminae 1, 3 and 5 of the inner plexiform layer, at least in human eye [15]. In the periphery it is an important part of the sensory system and exerts local immune and inflammatory responses in distinct microenvironments [21]. The eye nerves derive from the trigeminal ganglion and provide sensory supply, i.e. SP-ergic nerves, to most ocular tissues, in particular cornea, blood vessels, ciliary body and choroid [23]. Sensory transmitters are known to mediate the ocular response to various irritative stimuli [25].

In preliminary studies we found significantly elevated levels of certain neuropeptides in aqueous humor (AH) of patients with proliferative vitreoretinopathy (PVR), but
different grades of the disease were not considered [14, 24]. The peptide of current interest, SP, was highly concentrated in a distinct manner; both cataract controls and patients with retinal detachment (RD) or proliferative diabetic retinopathy featured normal low levels, thus emphasizing processes associated with PVR [14].

The purpose of the present study was to characterize the significance of these circumstances in more detail, i.e. detection of SP in AH was reproduced in RD and in PVR and extended to fundoscopic control examination in the RD group to investigate how the SP concentration at the time of RD surgery relates to subsequent development of PVR and to measurement of SP in various grades of PVR. Cataract patients served as controls.

### Patients, materials and methods

The study was formulated as a prospective study over a period of 12 months. Each patient suffering from rhegmatogenous RD or PVR who was under examination and/or treatment by one of the authors within this period was entered in the study. Thus, AH of 80 patients (43 males, 37 females; see Table 1) was analyzed. Fundoscopic examination using the three mirrors of the Goldmann contact glass in the RD group was performed on the day of admission, together with a detailed anamnesis, and again 6 months after primary surgical reattachment of the retina to recognize proliferative processes due to PVR. Each of the patients was receiving surgical treatment for the first time, no patient enrolled in our former study [14] was entered again in the present study and none of the patients had clinical signs of other diseases, in particular uveitis.

Forty-two patients underwent scleral buckling including cryocautery as a consequence of uncomplicated rhegmatogenous RD. The patients in this group included all subjects surgically treated by one surgeon within a 6-month period. Ten patients required vitrectomy, membrane peeling, gas or silicone oil tamponade for PVR P1, seven patients for PVR P2, six patients for PVR P3 and five patients for PVR P4–A4 (grading according to the Cologne Classification [10]). Twelve patients with PVR required surgery a second time and three patients a third time. One patient developed PVR P4–A4 3 months after primary surgical reattachment of the retina within the 6-month period and patients without surgery for RD in the fellow eye, and no patient was treated systemically with steroids or nonsteroidal anti-inflammatory drugs prior to surgical procedures.

Preoperatively, AH was aspirated by paracentesis (150 μl) and immediately frozen at −80°C. After collection of sufficient samples, AH samples were analyzed by radioimmunoassay without prior extraction. A total of 20 μl from the same sample was subjected to determination of the protein concentration using the Lowry method [19]. Radioimmunoassay for SP-IR was performed with a specific antiserum (RD2) and (125I)Bolton-Hunter SP as tracer. Incubation was performed for 48 h without and for an additional 48 h with the tracer added (about 15000 cpm; Amersham) as previously described [14]. Bound and free radioactivity were separated with a secondary antibody (GARGG-500) and normal rabbit serum (NRS-500; Peninsula). Under these conditions the detection limit was about 6 fmol per sample. The concentration of each sample was sorted according to disease type. Statistical calculation of differences between the disease categories was performed with the Mann-Whitney U-test.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Patients</th>
<th>Mean age (years)</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cataract</td>
<td>10</td>
<td>63.4/58.6</td>
<td>5/5</td>
</tr>
<tr>
<td>RD</td>
<td>42</td>
<td>31.9/36.5</td>
<td>23/19</td>
</tr>
<tr>
<td>P1</td>
<td>10</td>
<td>26.3/33.2</td>
<td>4/6</td>
</tr>
<tr>
<td>P2</td>
<td>7</td>
<td>45.5/33.4</td>
<td>2/5</td>
</tr>
<tr>
<td>P3</td>
<td>6</td>
<td>30.5/27.5</td>
<td>2/4</td>
</tr>
<tr>
<td>P4–A4</td>
<td>5</td>
<td>26/33.5</td>
<td>1/4</td>
</tr>
</tbody>
</table>

### Results

The concentrations of SP measured in each sample are summarized in Fig. 1a, and the mean values±SEM of various disease categories are illustrated in Fig. 1b. SP was detected in each sample; in no sample was it below the detection limit. The mean concentration of SP in control eyes with cataract was 40.3 (+22.4) fmol/mg protein and in eyes with rhegmatogenous RD 61.9 fmol/mg protein (±13.9, range from 11 to 558). Note the high values of 558, 236 and 198 fmol/pg protein in three RD patients (Fig. 1a). These patients developed PVR. At the date of control PVR was observed in a total of four patients of the RD group – those with enhanced levels of SP and one further patient who initially showed no elevation of SP. Nevertheless, values of subjects developing PVR were significantly higher than those not developing PVR (P<0.05).

In PVR P1, SP averaged 327.8 fmol/mg protein (±28.3, range 226–511), in PVR P2, 383.7 fmol/mg protein (±77.5, range 217–804), in PVR P3, 347.5 fmol/mg protein (±56.9, range 219–621) and in PVR P4–A4, 282.4 fmol/mg protein (±23.7, range 197–333). Analysis showed a statistically significant difference in mean concentrations between the RD group and the various PVR groups (P<0.001, Fig. 1b). No statistically significant difference was observed among the various PVR groups, but, instead, a peak in P2 and a decreasing tendency to P4–A4.

It must be emphasized that the currently used antisem were not considered [14, 24]. The peptide of current interest, SP, was highly concentrated in a distinct manner; both cataract controls and patients with retinal detachment (RD) or proliferative diabetic retinopathy featured normal low levels, thus emphasizing processes associated with PVR [14].

### Discussion

Our results show that SP levels in AH of patients with PVR are significantly higher than in cataract controls and patients with RD ([14] and Fig. 1a). Detailed analysis of the concentrations reveals two remarkable novel as-