Serotoninergic status in patients with hereditary vascular retinopathy syndrome

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Abstract

Aim/background—In a new autosomal dominant syndrome (which the authors called hereditary vascular retinopathy (HVR)) cerebral ischaemia, Raynaud’s phenomenon, and migraine are the most striking features. As serotonin (5-HT) is known to play a role in vasospastic processes, Raynaud’s phenomenon, and migraine they wondered whether the serotoninergic status in patients with HVR is different. Therefore, it was decided to investigate some serotoninergic variables in these patients.

Methods—The study was conducted in 12 patients with HVR, 10 relatives, and 19 healthy controls. The levels of intraplatelet and plasma 5-HT were measured, as well as the plasma levels of its precursor amino acid tryptophan and the ratio of tryptophan to the large neutral amino acids, which compete with the transport of tryptophan through the blood-brain barrier.

Results—In both the patients with HVR and in nine relatives the concentrations of 5-HT in plasma and platelets were significantly lower than in controls. The plasma levels of tryptophan and the tryptophan ratio were also found to be lower in the patient group compared with the control group, but not in the relatives.

Conclusion—The observed alterations in 5-HT and its precursor tryptophan strongly suggest the existence of a malfunctioning of the serotoninergic system in the HVR syndrome.

Hereditary vascular retinopathy (HVR) is characterised by retinal microangiopathy, cotton wool patches (oedema, swelling, and degeneration of the nerve fibres), retinal haemorrhages, and areas of capillary non-perfusion, and in more advanced stages occlusion of large retinal vessels causing large avascular areas which may induce a neovascular response (Fig 1). The retinopathy becomes symptomatic around the age of 40 years, giving a gradual decrease of visual acuity, transient visual disturbances, and visual field defects. At the age of 60 years, most patients experience severe visual impairment even up to blindness.

We examined a large pedigree spanning three generations showing an autosomal dominant pattern of inheritance. Apart from the retinopathy, a high prevalence of Raynaud’s phenomenon and migraine was found in this family. Moreover, mental symptoms such as loss of memory and aggressive behaviour were mentioned in some members of this family. Five out of seven HVR patients who were examined neurologically also showed cerebral vascular involvement. Since it is known that serotonin (5-HT) plays some role in vasospasm, Raynaud’s phenomenon, migraine, and aggression we decided to determine some serotoninergic variables in these patients.

5-HT is mainly synthesised in enterochromaffin cells of the gut. After release in the blood, 5-HT is taken up by the platelets or inactivated by the liver and the lungs. Consequently, the concentrations of 5-HT in plasma are rather low. It is assumed that 5-HT in plasma represents a distinct pool with a rapid turnover, different from 5-HT in platelets which represents a reserve pool with a slow turnover. Biodegradation and inactivation by the liver and the lungs. Consequently, the concentrations of 5-HT in plasma are rather low. It is assumed that 5-HT in plasma represents a distinct pool with a rapid turnover, different from 5-HT in platelets which represents a reserve pool with a slow turnover. Biodegradation and inactivation by the liver and the lungs. Consequently, the concentrations of 5-HT in plasma are rather low. It is assumed that 5-HT in plasma represents a distinct pool with a rapid turnover, different from 5-HT in platelets which represents a reserve pool with a slow turnover. Biodegradation and inactivation by the liver and the lungs.
neovascularisation causes hyperfluorescence. Several avascular areas are present. At the optic disc an incipient neovascularisation caused hyperfluorescence.

Figure 1 Right eye of a 53 year old man with hereditary vascular retinopathy (HVR) showing enlargement of the central avascular area. Microaneuroms and telangiectatic capillaries are located around the macula. A branch of the temporal inferior vein is showing enlargement of the central avascular area. Microaneurysms and telangiectatic degeneration of the nerve fibres, retinal haemorrhages, and areas of capillary non-perfusion. The study group consisted of first and second degree relatives and no systemic diseases were present in these subjects. Some of these subjects used non-steroidal anti-inflammatories, anti-histaminergic drugs, diuretics, paracetamol, and diazepam, and one subject used oral contraceptives. These drugs, except for oral contraceptives which may increase the plasma concentration of tryptophan, do not influence our biochemical determinations. The control subjects were free of medication. Both the patients and the healthy controls gave their written informed consent to the study after being informed about the aim and method of the investigation.

All blood samples (20 ml) were collected in September between 9 am and 11 am. Blood was drawn by antecubital puncture through a needle and collected in two siliconised Vacutainer tubes containing EDTA. Platelet rich plasma (PRP) was prepared by centrifuging the blood for 20 minutes at 90 g and plasma was obtained after an additional run at 2650 g for 20 minutes. Platelets were counted in both PRP and plasma; in the latter the number of platelets was always below 9 × 10⁸/ml, indicating negligible platelet contamination. The samples were frozen and stored at −80°C until analysis.

BIOCHEMICAL DETERMINATIONS

The 5-HT levels in both PRP and plasma were measured by high performance liquid chromatography (HPLC) and electrochemical detection, according to a modification of the method of Bax et al.⁰ The proteins were removed with 4.2% (w/v) trichloroacetic acid in the presence of methyl tryptophan as internal standard. Samples of 30 μl were injected onto a 125 × 4 mm Lichrospher 100 RP-18 5 μm column (Merck, Darmstadt, Germany), which was protected by a 4 × 4 mm guard column of the same material. The mobile phase was composed of 10 mM KH₂PO₄ pH 3.6, which contained 10% methanol and was delivered at a flow rate of 0.8 ml/min at 30°C. Detection was accomplished at 278 nm (10 nm bandwidth) using a Hewlett Packard multiple wavelength detector (reference wavelength 450 nm, bandwidth 80 nm). Recoveries for added tryptophan were 94% (5%) (mean (SD), n=10).

Other amino acids were determined using an LKB 4400 amino acid analyser with fluorescence detection.¹ The plasma tryptophan ratio was determined by dividing the plasma tryptophan concentration by the sum of the other large neutral amino acids—that is, valine, isoleucine, leucine, tyrosine, and phenylalanine. This ratio is a measure for the amount of tryptophan entering the brain, which is subsequently available for the synthesis of 5-HT.³

STATISTICAL ANALYSIS

All data are presented as mean (SD). Differences between the patient groups and the control group were assessed by one way analysis of variance (ANOVA). The Bonferroni multiple comparison test was selected as post-test to compare pairs of group means. The minimum level of significance was considered as p<0.05 (two tailed).

Results

All serotoninergic variables measured differed between the patient groups and the control group (ANOVA; p values ranged between <0.001 and 0.0138). The p values and F ratios for each variable are shown in Table 1. With the Bonferroni test for individual variables, it was found that the plasma 5-HT concentration in the 12 HVR patients (5.6 (1.4) nmol/l) was significantly lower than in the controls (9.8 (2.8) nmol/l, p<0.001; Table 1 and Fig 2). In the relatives, except for one, the plasma 5-HT level was also decreased (3.1 (1.1) nmol/l; p<0.001). The platelet 5-HT contents in HVR patients and relatives (2.2 (0.8) and 2.0 (0.9)
Table 1: Biochemical variables in hereditary vascular retinopathy (HVR) patients, relatives, and healthy controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n=19)</th>
<th>HVR patients (n=12)</th>
<th>Relatives (n=9)</th>
<th>One way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p value</td>
</tr>
<tr>
<td>Plasma 5-HT (nmol/l)</td>
<td>9.8 (2.8)</td>
<td>5.6 (1.4)*</td>
<td>5.1 (1.1)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet 5-HT (nmol/10⁹ platelets)</td>
<td>3.2 (0.6)</td>
<td>2.2 (0.8)*</td>
<td>2.0 (0.9)*</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plasma tryptophan (µmol/l)</td>
<td>51 (8)</td>
<td>42 (6)*</td>
<td>45 (6)</td>
<td>0.0024</td>
</tr>
<tr>
<td>Tryptophan ratio</td>
<td>8.7 (0.9)</td>
<td>7.7 (1.1)*</td>
<td>7.8 (1.0)</td>
<td>0.0138</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD).
*Significantly different from control group (p<0.001); †Significantly different from control group (p<0.01); ‡Significantly different from control group (p<0.05; Bonferroni test).

As mentioned, one relative showed a deviating plasma 5-HT level (30.5 nmol/l). This woman also differed from the other relatives in plasma tryptophan level (57 µmol/l). One year later another blood sample was taken and the plasma was reanalysed. The concentration of 5-HT was even higher in this sample than the year before.

**Discussion**

Serotonin is known to be involved in the pathogenesis of Raynaud’s phenomenon and migraine, and it also plays a role in vasoconstriction.

In the HVR syndrome occlusion occurs in the retinal and possibly the cerebral vessels. Moreover, 80% of the patients with the retinopathy suffer from Raynaud’s phenomenon and 60% suffer from migraine. In the family studied, plasma 5-HT, platelet 5-HT content, and plasma tryptophan levels are decreased; not only did the HVR patients show these abnormalities but also the relatives studied. The low plasma levels of 5-HT in the study group are probably not due to the occurrence of migraine, because the subjects without migraine had the same plasma 5-HT concentrations as those with migraine. Since six of the eight relatives with these abnormalities suffered from migraine or Raynaud’s phenomenon (one subject even suffered from both conditions), it is possible that these people—being also younger than the HVR patients—are still at risk of developing the retinopathy in future, indicating that these decreased biochemical variables are trait markers.

Several mechanisms may account for these abnormal values: (1) malabsorption of tryptophan in the intestinal mucosa, (2) increased conversion of tryptophan into the coenzyme nicotinamide, acetyl CoA, and acetoacetyl CoA (the kynurenine pathway), (3) increased utilisation of 5-HT and consequently of tryptophan, and (4) increased excretion of tryptophan. There were no indications of low dietary tryptophan intake in these patients. Moreover, it has been suggested that food intake does not affect plasma and whole blood 5-HT. The exact mechanism for the observed differences cannot yet be determined.

In conclusion, we found a derangement in the serotoninergic system of patients suffering from HVR and in their relatives. Whether
5-HT is pathophysiologically or aetiologically involved in vascular retinopathy or the abnormal 5-HT status in this syndrome is an epiphenomenon requires further investigation.

7 Humphrey PPA. 5-Hydroxytryptamine and the pathophysiology of migraine. *J Neurol* 1991;238:S38–44.