

Serotonergic 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 serotonergic status in patients with HVR is different. Therefore, it was decided to investigate some serotonergic 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 serotonergic system in the HVR syndrome.

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Hereditary vascular retinopathy (HVR) is characterised by retinal microangiopathy, cotton wool patches (oedema, swelling, and degeneration of the nerve fibres), retinal haemorrhages, 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).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.² 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.² Since it is known that serotonin (5-HT) plays some role in vasospasm,^{3,4} Raynaud's phenomenon,^{3,5} migraine,⁶⁻⁸ and aggression⁹ we decided to determine some serotonergic 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.^{10,11} 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.¹² Biondi *et al*⁵ reported that 5-HT was significantly higher in plasma and platelets from Raynaud's patients than from normal controls. These authors concluded that their data indicate that these patients have increased circulating 5-HT. On the other hand, Ferrari *et al*⁸ reported that migraine patients have chronically low systemic 5-HT. Between attacks, plasma levels of 5-HT were about 60% lower compared with healthy control subjects, while platelet 5-HT levels were normal. Besides, no changes were demonstrated for 5-HT precursor plasma levels.

Because of the above mentioned frequent occurrence of Raynaud's phenomenon and migraine in HVR, we wondered what the serotonergic status in these patients would be. For this reason, we determined the concentrations of 5-HT in blood platelets and plasma, the plasma concentration of its precursor, the amino acid tryptophan, and the ratio of tryptophan to the other large neutral amino acids. The latter compete with tryptophan for the transport carrier at the blood-brain barrier. This tryptophan ratio is a measure of the amount of 5-HT synthesised in the brain,¹³ which may correlate with the incidence of aggression.

Subjects and methods

We studied 12 patients suffering from HVR (seven females, five males, aged 47 (SD 12) years), 10 relatives (five females, five males, aged 36 (8) years), and 19 controls (nine females, 10 males, aged 36 (6) years), healthy individuals from among the hospital staff. The criteria for diagnosis of HVR were the presence of funduscopic and/or angiographic abnormalities—that is, microangiopathy, cotton wool patches (oedema, swelling, and

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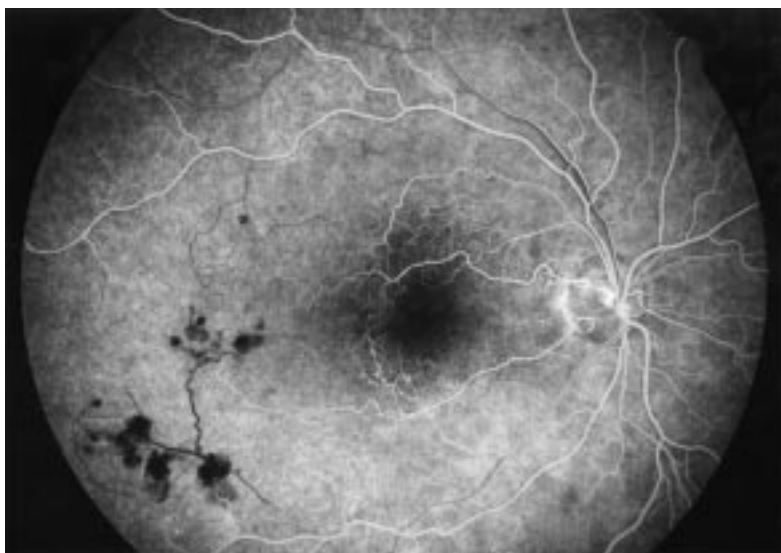


Figure 1 Right eye of a 53 year old man with hereditary vascular retinopathy (HVR) showing enlargement of the central avascular area. Microaneurysms and telangiectatic capillaries are located around the macula. A branch of the temporal inferior vein is occluded. Several avascular areas are present. At the optic disc an incipient neovascularisation causes hyperfluorescence.

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 antiinflammatories, antihistaminergic 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 9×10^9 /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.*¹⁴ The proteins were removed with 4.2% (w/v) sulphosalicylic acid after the addition of the internal standard isoprenaline. Samples of 20 μl (PRP) or 40 μl (plasma) were injected onto a reversed phase column (CP-Spher-C8, 5 μm particle size, 200 \times 3 mm, Chrompack, Middelburg, Netherlands) which was protected by a guard column (2.1 \times 3 mm)

of the same material. The mobile phase consisted of 75 mM sodium acetate, 0.27 mM disodium EDTA, 2.13 mM heptane sulphonic acid, and 20% methanol, pH 4.15. The flow rate was set at 0.4 ml/min and the column temperature was 40°C . The detection system consisted of a Model 5100A Coulochem detector equipped with a 5021 conditioning cell and a 5011 high sensitivity cell (ESA, Bedford, MA, USA). The potentials for the conditioning cell and detectors 1 and 2 were -0.06 , $+0.03$ and $+0.48$ V, respectively (gain 10×100). The detector was linked to a HP 3396A integrator (Hewlett Packard) and quantification was done by measuring peak heights. The recovery of 5-HT added to the plasma samples was 90–98%.

Tryptophan was determined by HPLC after deproteinating the plasma with 3% (w/v) trichloroacetic acid in the presence of methyl tryptophan as internal standard. Samples of 30 μl were injected onto a 125 \times 4 mm Lichrospher 100 RP-18 5 μm column (Merck, Darmstadt, Germany), which was protected by a 4 \times 4 mm guard column of the same material. The mobile phase was composed of 10 mM KH_2PO_4 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.¹⁵ 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.¹³

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 serotonergic 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 (5.1 (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

Variables	Controls (n=19)	HVR patients (n=12)	Relatives (n=9)	One way ANOVA	
				p value	F ratio
Plasma 5-HT (nmol/l)	9.8 (2.8)	5.6 (1.4)*	5.1 (1.1)*	<0.0001	20.91
Platelet 5-HT (nmol/10 ⁹ platelets)	3.2 (0.6)	2.2 (0.8)*	2.0 (0.9)†	0.0001	11.84
Plasma tryptophan (μmol/l)	51 (8)	42 (6)†	45 (6)	0.0024	7.11
Tryptophan ratio	8.7 (0.9)	7.7 (1.1)‡	7.8 (1.0)	0.0138	4.82

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).

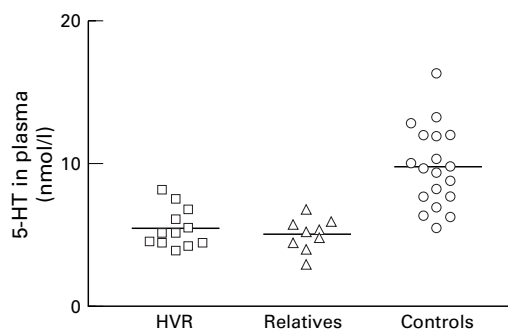


Figure 2 Scatter plots of serotonin (5-HT) levels in the plasma of hereditary vascular retinopathy (HVR) patients, relatives, and controls. For each group the individual levels and mean level are shown.

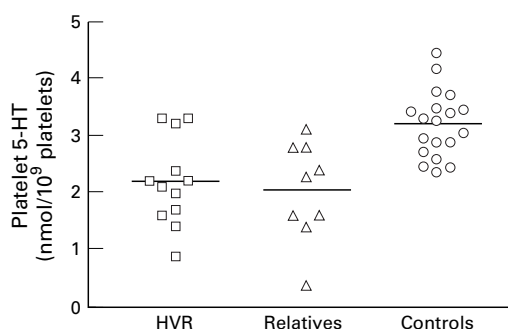


Figure 3 Scatter plots of intraplatelet contents of serotonin (5-HT) in hereditary vascular retinopathy (HVR) patients, relatives, and controls. For each group the individual levels and mean level are shown.

nmol/10⁹ platelets, respectively) were also significantly lower than in the control group (3.2 (0.6) nmol/10⁹ platelets, p<0.01 and p<0.001, respectively; Table 1 and Fig 3).

The mean plasma tryptophan concentration in the 12 investigated patients was also significantly lower than in the control group (42 (6) μM *v* 51 (8) μM, p<0.01). Although the tryptophan concentration in the relatives (45 (6) μM) was slightly higher than in the patients, it was still lower than in the controls, although no significance was reached (Table 1 and Fig 4). Comparable differences in plasma tryptophan ratios between the patient groups and controls were found. This ratio was also only significantly lower in the HVR patients compared with the control group (7.7 (1.1) *v* 8.7 (0.9), p<0.05; Table 1). None of the other amino acids measured differed significantly between the groups studied. There was also no difference between the plasma 5-HT level in the patients with migraine and in the subjects not suffering from migraine (5.6 (1.6) and 5.1 (1.1) nmol/l, respectively).

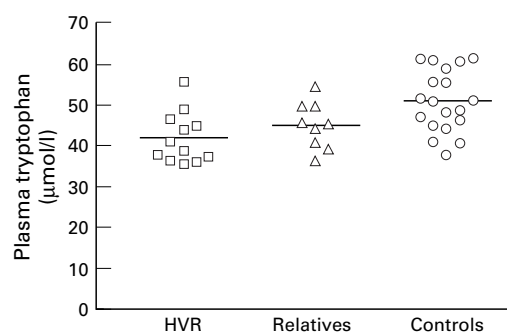


Figure 4 Scatter plots of tryptophan concentrations in the plasma of HVR patients, relatives, and controls. For each group the individual levels and mean level are shown.

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. Therefore, her biochemical variables were not taken into account in the analyses.

Discussion

Serotonin is known to be involved in the pathogenesis of Raynaud's phenomenon^{3 5 16} and migraine,⁶⁻⁸ and it also plays a role in vasoconstriction.^{3 4}

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 kynurenin 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.¹² The exact mechanism for the observed differences cannot yet be determined.

In conclusion, we found a derangement in the serotonergic 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.

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