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Serotonin (5-HT) and the rat's eye

Some pilot studies

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Abstract. Serotonin (5-hydroxytryptamine, 5-HT) is a biogenic amine which has a multitude of more or less clearly established effects on peripheral vessels. It influences blood viscosity, platelet aggregation, and vasoconstruction and -dilatation, it enhances capillary permeability, it is the precursor of melatonin (a hormone with diurnal production in the eye). Because of these actions a role for serotonin in the development of glaucoma and diabetic retinopathy might be suspected. In a series of pilot studies on rats the effects of serotonin on the anterior and posterior segments of the eye were studied. Serotonin had marked influence on the retinal and choroidal vasculature. The optic disk seemed to be very sensitive to serotonin. Possibly it had an influence on the blood-retinal barrier. It caused transient cataracts, probably by decreasing the production of aqueous. It blocked tropicamin-induced mydriasis. The techniques and provisional results of measurement of serotonin in human aqueous are also described.

Introduction

Serotonin (5-Hydroxytryptamine, 5-HT) is a biogenic amine which exhibits a multiplicity of pharmacological actions. Because of the existence of multiple receptor sites, the physiological role of 5-HT has not yet been clearly established and the results of different studies are often contradictory or controversial [1, 2]. 5-HT is mainly produced in the enterochromaffin cells of the small intenstine. It is either removed by the liver and the endothelium of the lungs or it is taken up by blood-platelets, which release it when they aggregate [3]. 5-HT is also synthesized in the central nervous system, where it acts as a neurotransmitter. Furthermore, 5-HT is the precursor of melatonin, a hormone which is produced in both the eyes and the pineal gland (third eye) [4]. Melatonin has an influence on retinal disk shedding [5] and on various endocrinological systems, through which a relationship with intra-ocular pressure and proliferative diabetic retinopathy might be suspected.

5-HT has an influence on haemorheology. It increases blood viscosity [6] and plays a role in platelet aggregation [7]. It has a complex influence on vasoconstriction and vasodilatation [8, 9]. Furthermore, it enhances peripheral microvascular permeability and thus plays a role in the develop-

ment of oedema [10]. These actions of 5-HT suggest a possible relationship between 5-HT and diabetes mellitus. There is evidence that 5-HT interacts with the glucose and insulin metabolism [11–14]. The reactions of the vessels to 5-HT in diabetic animals differ from those of healthy animals [15]. Winocour et al. [16] recently described significantly higher plasma concentrations in diabetic patients than in healthy subjects.

Because of the diversity of, mainly unclarified, actions on the general circulation and microcirculation, 5-HT might play a role in several eye diseases in which the circulation is involved, i.e. diabetic retinography, arterial and venous occlusion and glaucoma. The reaction of the retinal vessels to 5-HT was first described in 1959 by Morlunghi & Volpi [17], followed by Tammisto [18], who described the transient blanching of the fundus after intravenous injection of 5-HT. A more detailed study by Okhubo & Chiba [19, 20] showed the constrictive action on perfused canine ophthalmic arteries and human ciliary arteries. Williams et al. [21] and Faraci et al. [22], however, found no alteration in retinal bloodflow after 5-HT in healthy monkeys, whereas in atherosclerotic monkeys there was a marked reduction in bloodflow and a decrease in the reaction to light stimuli on the ERG. In a double-blind study on the influence of an anti-5-HT drug on the progression of non-proliferative diabetic retinopathy, Klein & Hirche [23] found significantly less progression in retinal haemorrhages and microaneurysms in the treated group.

In consideration of the vasoactive properties of 5-HT and the fact that both intra-ocular pressure [24, 25] and melatonin (metabolite of 5-HT) production follow a diurnal rhythm in the eye, there is reason to suspect a possible role for serotonin in the development of glaucoma. Martin et al. [26, 27] were the first to measure 5-HT in human aqueous and speculated on its mode of action in glaucoma. In a double blind study on patients with normal tension glaucoma, Mermoud et al. [28] found a positive influence of naftidrofuryl (an anti-5-HT drug) on visual acuity and visual field, even though the intra-ocular pressure was slightly raised during treatment. Another anti-5-HT drug, ketanserin, was found to lower the intraocular pressure significantly in patients with ocular hypertension [29].

The enigmatic features of 5-HT listed above, induced the authors to conduct a series of pilot-studies. 5-HT was given in various doses by various methods of administration to healthy pigmented rats. The effects on the posterior and anterior segments were studied by ophthalmoscopy, fundus-photography, fluorescein-angioscopy, fluorescein-angiography, slit-lamp examination and photography. Meanwhile, laboratory techniques for measuring 5-HT in human aqueous were developed.

Materials and methods

Throughout the pilot studies healthy pigmented AO × BN rats, three to six months of age, were anaesthetized with 0.25 ml Aescoket i.m. and 0.1 ml

Rompun s.c. after superficial ethersedation. 5-HT solutions of various concentrations were prepared by dissolving 5-HT-creatinine sulphate (Sigma) in either saline or phosphate buffer solution (PBS). Care was taken to expose the solutions as little as possible to light. Fluorescein-angioscopy and -angiography were performed on some rats after intraperitoneal injection of 0.3 ml of a 5% fluorescein solution. A Zeiss fundus camera was used.

Experiment 1: posterior segment

(a) *Peri-ocular injections:* after lifting the conjunctiva an injection of 0.05 ml of a respectively 1, 0.5, 0.25 and 0.01 mg/ml 5-HT solution in PBS was given to the right eye, the left eye served as the control eye. Mydriasis was obtained by using tropicamide 1% eyedrops prior to the injections. The effects were studied both ophthalmoscopically and by fluo-angioscopy and -angiography.

(b) *Intraperitoneal injections:* 0.2, 0.1 and 0.02 mg 5-HT in 0.5 ml PBS were injected. Effects in both eyes were examined.

(c) *Eyedrops:* 1% solutions of 5-HT in saline and in PBS were prepared and instilled into the right conjunctival sac immediately after anaesthesia and 5 and 10 minutes later. The left eye served as the control eye.

Experiment 2: anterior segment

(a) After the injections and eyedrops as described in Experiment 1 the anterior segments of some rats were studied by slit-lamp examination.

(b) After intraperitoneal injection the penetration of fluorescein into the anterior chamber was examined.

(c) The effects on mydriasis were studied by administration of 1% 5-HT eyedrops immediately after anaesthesia and 5 and 10 minutes later into the conjunctival sac of either the right or left eye, the contralateral eye served as control. After 15 minutes tropicamide 1% eyedrops were instilled into the conjunctival sac of both eyes.

Experimental 3: 5-HT in human aqueous

5-HT concentrations were measured in the aqueous of 5 patients who were scheduled for ECCE and IOL implantation under local anaesthesia and were not known to have either glaucoma or diabetes mellitus. Retrobulbar anaesthesia consisted of 2.5 ml Lidocaine 2% and 2.5 ml Marcaine 0.5%. Mydriasis was effected by means of tropicamide 1% and phenylephrine 2.5% eyedrops. Using a 30G needle on a 1 ml syringe, 0.15 ml aqueous was aspirated from the anterior chamber, prior to the opening of the conjunctiva. Samples were conserved by the addition of sodium metabisulphite and the disodium salt of ethylenediaminetetraacetate to a final concentration of approximately 10 g/l each. Samples were stored, protected from light, at $-20°$ C until analysis. 100 µl aliquots were analyzed by direct injection on a

high performance liquid chromatography system with fluorometric detection, as described by Kwarts et al. [30].

Results

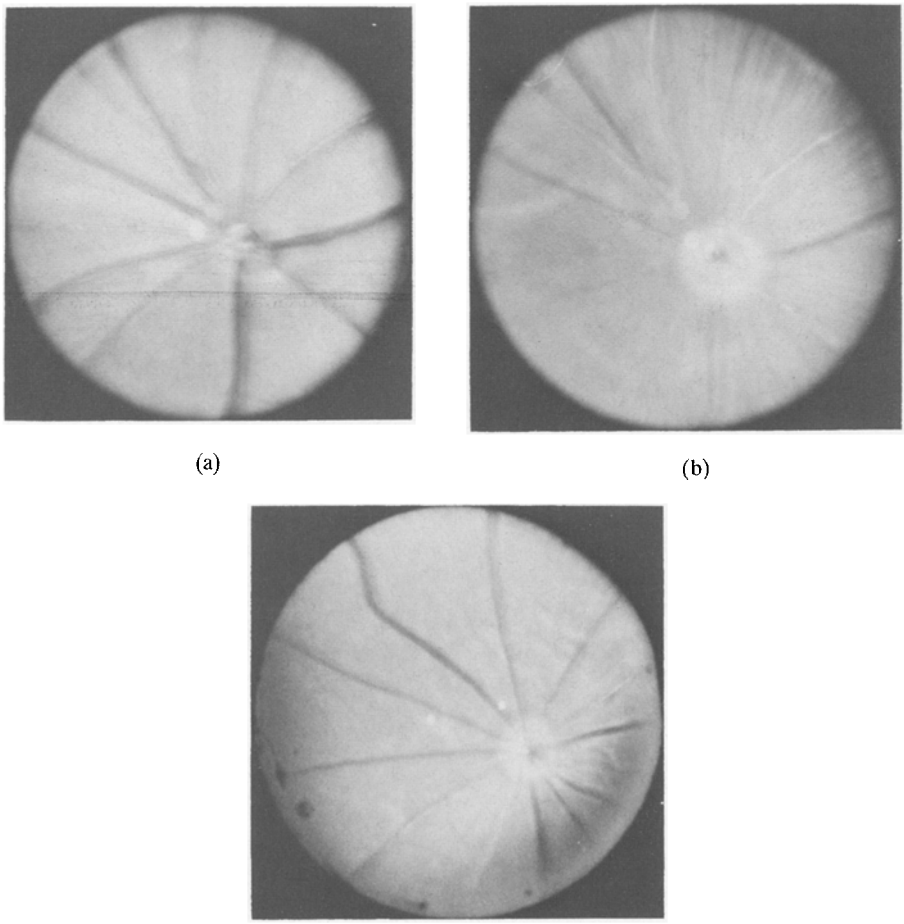
Experiment 1

As shown in Fig. 1a & 1b, peri-ocular injection of a 1 mg/ml 5-HT solution caused marked attenuation of the vessels. After 1 minute blanching of the optic disk could be observed, followed by blanching of the retinal arterioles and venules and of the choroidal vasculature. The retina was left virtually bloodless. The effect was temporary. In some rats it took 20 minutes before the fundus was perfused again, in others it took 1 hour or more. Rats that were re-examined the day after the experiment all had normal-looking fundi. When 5-HT in lower concentrations was used, the effects were less pronounced. As shown in Fig. 1c the vessels became less attenuated, though occasionally some sludging was seen. An important finding was that, even when there was no macroscopic narrowing of the retinal vessels after these low concentration injections, there was still marked blanching of the optic disk.

Because the findings might possibly be caused by the local effects of the injection in the peri-ocular region, i.e. haemorrhage or oedema, the experiments were repeated using intraperitoneal injection of 5-HT. The effects were comparable. Injection of 0.2 mg 5-HT i.p. caused marked attenuation within 2 minutes. After a 0.05 mg injection there was still marked paleness of the optic disk. As shown in Fig. 2, the effects of these low-dose injections were comparable to those of 1% 5-HT eyedrops. Though the effects of eyedrops were not observed in every animal, in some there was a definite blanching of the optic disk.

Because the 5-HT injections caused fast-developing cataracts it was hard to obtain reproducible fluorescein angiograms. Though the larger vessels were well documented, it was hard to collect data on the capillary system. However, after injections of medium to high doses of 5-HT, when ophthalmoscopically there was complete closure of the retinal vascular system, some filling of the arteries could be observed, while the venous system did not fill at all. This suggests closure of either the capillary system or the veins. After low-dose injections, when opthalmoscopically the optic disk was very pale, some fluoresein still entered the disk, suggesting either partial closure of all disk vessels, or closure of either the retinal or the choroidal/ciliary blood supply.

Furthermore, during fluorescein-angioscopy the impression arose that, when fluorescein was given after the vessels reopened, some leakage of dye from the capillaries could be observed. However, this impression could not be documented angiographically.



(a) (b)

(c)

Fig. 1. (a) Normal rat fundus; (b) Same fundus after peri-ocular injection of 0.05 ml 1 mg/ml 5-HT, showing blanching of the optic disk and marked attenuation of retinal and choroidal vessels; (c) Rat fundus after periocular injection of 0.05 ml 0.1 mg/ml 5-HT, showing less attenuation of the vessels, but still marked blanching of the optic disk.

Experiment 2

Both injections of 5-HT and eyedrops caused dense cataracts. After ten minutes an increasing opacification of the lens was observed. On slit-lamp examination a cortical cataract was seen behind a small clear subcapsular zone. In rats undergoing fluorescein angiography, no fluorescein was seen entering the anterior chamber after high- or medium-dose 5-HT injections; in rats that were given eyedrops there was a marked reduction in the fluorescence of the anterior chamber in comparison with the contralateral eye.

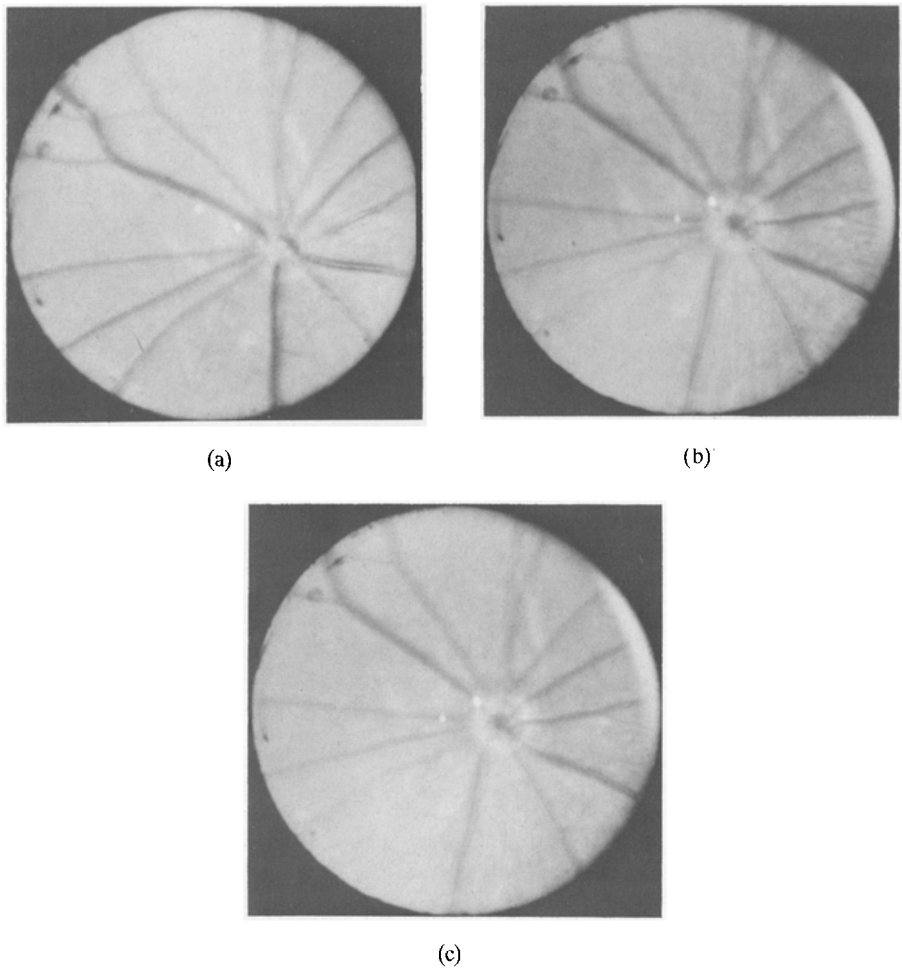


Fig. 2. (a) Normal rat fundus; (b) Same fundus after intraperitoneal injection of 0.5 mg 5-HT, showing paleness of the optic disk; (c) Rat fundus after 1% 5-HT eyedrops, showing paleness of the optic disk as in low-dose intraperitoneal injections.

When rats were given eyedrops in one eye immediately after anaesthesia, the pupil of this eye failed to dilate on administration of tropicamide 1% eyedrops. Because after 10 minutes of anaesthesia most rats showed a difference in spontaneous pupil dilatation (in most cases the left eye was more dilated than the right eye), the experiment was repeated on the eye that had the spontaneously larger pupil. In these cases also the pupil failed to dilate.

Experiment 3

In 3 of the 5 samples, a measurable concentration of 5-HT in the human aqueous was found. Concentrations were 4, 20, and 48 nmol/L respectively.

Discussion

As described by Tammisto [18], marked attenuation of the retinal vessels is observed after intravenous injection of 5-HT. The same observation was made in these pilot studies when 5-HT was given by the peri-ocular or intraperitoneal route. The reaction was dose-dependent. In high doses the retina became virtually bloodless. In lower doses, for instance when 5-HT was given in eyedrops, there was still marked blanching of the optic disk, even when the retinal vessels appeared to be unaffected. The optic disk vasculature seemed to be particularly sensitive to 5-HT. Whether the effect observed on ophthalmoscopy is due to actual spasm of the vessels or to obstruction of the vessels by cellular aggregates is still a debatable point. Other than in Tammisto's study, aggregates of blood corpuscles and sludging were seen in many of the experiments. On the other hand, the experiments of Okhubo and Chiba, although performed on another species, do point in the direction of actual spasm.

Although a fluorescein-angiogram of the rat's eye seems to be easily obtainable, efforts made to obtain reproducible data on the exact action of 5-HT on the retinal vessels and capillary network were unsuccessful, partly due to fast-developing cataracts. Some observations could be made, however. After a gross spasm of the retinal vessels some filling of the arteries could be observed but no filling of the veins. Whether this observation is explained by closure of the capillary network or by occlusion of the veins is not yet clear.

During fluorescein-angioscopy the impression of mild leakage of dye from the capillary system was obtained. Whether this observation is in fact a case of 'wishful looking' is not yet clear. Angiographic techniques are being improved and fluorophotometric studies are in progress, with the aim of documenting the suspected leakage of dye into the vitreous after 5-HT administration.

The influence of 5-HT on the rat's lens has been described by Tilgner & Kusch [31]. Cataracts were found when a subcutaneous injection of 5-HT was combined with a previous injection of phenelzine (a MAO-inhibitor). However in our experiments, when 5-HT was given alone by peri-ocular or intraperitoneal injection or in eyedrops, dense cataracts were observed as well. Possible explanations for these contradictory finding are that either the subcutaneous injection alone gives a very low blood concentration which is increased by phenelzine, or the anaesthetics used in our experiments play a role in potentiating the effects of 5-HT. The fact that the in-vitro study performed by Tilgner and Kusch showed a definite opacification of the isolated rat's lens on 5-TH alone, together with the finding that 5-HT eyedrops cause opacification, supports the view that 5-HT alone is responsible for the cataract formation. The cause of this cataract formation after 5-HT administration may be found in a disturbance of lens-metabolism due to diminishing bloodflow to the ciliary body and anterior chamber. As

shown in the experiments, fluorescein penetration into the anterior chamber was greatly diminished after 5-HT administration.

No reports have been found in the literature on the effects of 5-HT on pupillary dilatation. 5-HT blocked the dilatation of the pupil induced by tropicamide 1% eye-drops. Possibly this effect was due to hypoxia or ischaemia of the dilator muscle. A more sophisticated explanation may be found in a direct interaction between 5-HT and the autonomic nervous system [32]. This effect of 5-HT might play a role in the poor mydriasis observed in traumatic hyphaema: in hyphaema the concentration of 5-HT in the anterior chamber will be high because of its depletion from the platelets. It might also play a role in the poor mydriasis of diabetic patients. Studies are in progress to obtain more detailed information on this particular action of 5-HT.

As for the concentrations of 5-HT in human aqueous in this pilot study: they were although lower, still in the same order (nmol/mL) as those found by Martin et al. [26], and definitely higher than the concentrations found in the plasma of healthy people [16].

In conclusion, this series of pilot studies showed the multiple effects produced by 5-HT on the rat's eye. The results, together with the data found in the literature, support the hypothesis that 5-HT might play a role in the development of glaucoma and diabetic retinopathy. Angiographic techniques are being improved in order to get more insight into the action of 5-HT on the capillaries of the retina and optic disk. Fluorophotometric studies on healthy and Alloxan-diabetic rats are in progress. Measurements of 5-HT in human aqueous will be continued in healthy patients and in patients with glaucoma or diabetes. A study in a group of carcinoid patients will be undertaken [33]. Finally, the influence on mydriasis will be studied in more detail. Hopefully, in the future, these studies will shed more light on the interesting actions of 5-HT on the eye.

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References

1. Saxena PS. 5-Hydroxytrypramine receptors: functional responses and potential clinical uses of agonist and antagonist drugs. J Drug Res 1989; 14: 6–8.
2. Houston DS, Vanhoutte PM. Serotonin and the vascular system: role in health and disease, and implications for therapy. Drugs 1986; 31: 149–63.
3. Vanhoutte PM. Introductory remarks: why 5-hydroxytryptamine? Federation Proc 1983; 42: 211–12.
4. Zawilska JB, Nowak JZ. Regulatory mechanisms in melatonin biosynthesis in retina. Neurochem Int 1992; 20: 23–36.

5. LaVail MM, Lord BAP, Yasumara D. Circadian photoreceptor outer segment disc shedding in the rat. In: O'Brien PJ, Klein DC, ed., Pineal and retinal relationships. Academic Press 1986: 93–106.
6. Dormandy JA. Haemorheological aspects of serotonin. Prog Appl Microcirc 1986; 10: 62–75.
7. Swank RL, Fellman JH, Hissen WW. Aggregation of bloodcells by 5-hydroxytryptamine. Circ Res 1963; 13: 392–400.
8. Van Nueten JM. 5-hydroxytryptamine and the precapillary vessels. Federation Proc 1983; 42: 223–27.
9. Vanhoutte PM. Vascular effects of platelet released serotonin. Cardiovasc Drugs Ther 1990; 4: 7–12.
10. De Clerck F, Reneman RS. Serotonin and microvascular permeability. Prog Appl Microcirc 1986; 10: 32–43.
11. Leedom LJ, Meehan WP. The psychoneurendocrinology of diabetes mellitus in rodents. Pchychoneurendocrinology 1989; 14: 275–94.
12. Juszkiewicz M. The effect of insulin on the central serotonergic system of the rat. Pol J Pharmacol Pharm 1985; 37: 591–600.
13. Curzon G, Kantmaneni BD, Callaghan N, Sullivan PA. Brain precursors and metabolites in diabetic keto-acodosis. J Neurol Neurosurg Psychiatry 1982; 45: 489–93.
14. Lackovic Z, Salkovic M, Kuci Z, Relja M. Effect of long-lasting diabetes mellitus on rat and human brain monamines. J Neurochem 1990; 54: 143–47.
15. Mayhan WG. Impairment of endothelium-dependent dilatation of cerebral arterioles during diabetes mellitus. Am J Physiol 1989; 25: H 621–25.
16. Winocour PH, Klimiuk P, Grennan A, Baker RD, Weinkove C. Platelet and plasma vasoactive amines in type I diabetes mellitus with our without vascular disease. Ann Clin Biochem 1990; 27: 238–43.
17. Morlunghi C, Volpi U. Aziona della serotonina sui vasi oculari in coniglie normali, castrate e gravide. Ann Ottalmol Clin Oculista 1959; 85: 453–60.
18. Tammisto T. The effect of 5-hydroxytryptamine (serotonin) on retinal vessels of the rat. Acta Ophtalmol 1965; 43: 430–33.
19. Ohkubo H, Chiba S. Responses of isolated canine ophthalmic and ciliary arteries to vasoactive substances. Jpn J Ophthalmol 1987; 31: 627–34.
20. Ohkubo H, Chiba S. Vascular reactivities of isolated and perfused human ciliary arteries. Jpn J Ophthalmol 1988; 32: 450–56.
21. Williams JK, Baumbach GL, Armstrong ML, Heistad DD. Hypothesis: vasoconstriction contributes to amaurosis fugax. J Cereb Blood Flow Metab 1989; 9: 111–16.
22. Faraci FM, Williams JK, Breese KR, Armstrong ML, Heistad DD. Atheroscelerosis potentiates constrictor responses of cerebral and ocular blood vessels to thromboxane in monkeys. Stroke 1989; 20: 242–47.
23. Klein M, Hirche H. Naftidrofuryl in der Behandung der einfachen diabetischen Retinopathie. Eine Doppelblindstudie. Klin Mbl Augenheilk 1985; 187: 195–201.
24. Ericson LA. Twenty-four hourly variations of the aqueous humor flow. Acta Ophthamol 1958, suppl 50.
25. Smith SD, Gregory DS. A circadian rhythm of aqueous flow underlies the circadian rhythm of IOP in NZW rabbits. Invest Ophthalmol Vis Sci 1989; 30: 775–78.
26. Martin XD, Bernnan MC, Lichter PR. Serotonin in human aqueous humor. Ophthalmology 1987; 95: 1221–26.
27. Martin X. Mode d'action des beta-bloqueurs. Ophthalmologie 1990; 4: 154–55.
28. Mermoud A, Faggioni R, Van Melle GD. Double-blind study in the treatment of normal tension glaucoma with naftidrofuryl. Opthalmologica 1990; 201: 145–51.
29. Costagliola C, Scibelli G, Fasano ML, Ferrara LA, Mastropasqua L. Effect of oral ketanserin on intraocular pressure in glaucomatous patients. Exp Eye Res 1991; 52: 507–10.
30. Kwarts E, Kwarts J, Rutgers H. A simple paired-on liquid chromatography assay for

serotonin in cerebrospinal fluid, platelet rich plasma, serum and urine. Ann Clin Biochem 1984; 21: 425–29.
31. Tilgner S, Kusch T. Untersuchungen uber reversibele Linsentrubungen bei Ratten und Mausen nach kombinierter Applikation von Phenelzin un Serotonin. Opthalmologica 1969; 159: 211–22.
32. Humphrey PPA, Feniuk W, Watts AD. Prejunctional effects of 5-hydroxytryptamine on noradrenergic nerves in the cardiovascular system. Federation Proc 1983; 42: 218–22.
33. Wong VG, Melmon KL. Ophthalmic manifestations of the carcinoid flush. N Eng J Med 1967; 277: 406–9.

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