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Intraocular pressure variability in the anesthetized rat: a spectral analysis

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PURPOSE. *Intraocular pressure (IOP) is a measure of the balance between the inflow and outflow of the aqueous humor, being in close relationship with the venous ocular blood flow. But the influence of the autonomic nervous system upon this variable is not well understood. One of the most frequently used mathematical tools for the evaluation of the autonomic nervous system in the frequency domain is the fast Fourier algorithm (FFT) applied to the analysis of heart rate (HR) and arterial blood pressure (BP). For these variables, a power spectrum has been built showing the major bands: very low frequency, lower frequency, and higher frequency (HF). The range of these bands depends on the animal species. In this study, the authors used FFT to analyze the variability of IOP in anesthetized rats.*
METHODS. *BP and electrocardiogram were acquired at 2 KHz in all animals before and following muscle blockade and artificial ventilation at the same frequency as the spontaneous ventilation. Also, in this last condition, IOP was recorded before and after the application of atropine in the eye.*
RESULTS. *Results show three bands in the IOP spectrum, a similar profile to those observed in the HR and BP spectra, with HF band modified after atropine application*
DISCUSSION. *The discussion calls attention to the influence of the autonomic nervous system on IOP and suggests the possibility of clinical application of this methodology on diagnosis and therapeutic efficacy. (Eur J Ophthalmol 2004; 14: 381-6)*

KEY WORDS. *Intraocular pressure, Spectral analysis, Autonomic nervous system, Fast Fourier transform, Variability, Dysautonomy, Pathology*

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INTRODUCTION

Intraocular pressure (IOP), one of the factors by which the eye maintains its shape, is continuously determined by the relation between the amount of aqueous humor produced in the ciliary process and its rate of drainage to the venous circulation via the Schlemm's canal and trabecular meshwork. Physiologic values for IOP range from 10 to 21 mmHg with fluctuations of 1 mmHg correlating with pulsations in venous blood pressure (BP) (1). In the human subject, the production and drainage of aqueous humor shows a diurnal variation, the lower level in the morning and the higher in the afternoon (1), and is exaggerated in patients with glaucoma (2). The inflow and the outflow of aqueous humor is in close relation with the vascular system, itself under influence of the autonomic nervous system, but clear evidence of autonomic control of IOP has not been achieved (1, 3). Studies in rabbits have shown a similar circadian vari-

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ation of IOP, which was affected by cervical sympathectomy that evoked a decrease in the overall levels of IOP (4). Also, stimulation of superior cervical ganglion of the rabbit decreased IOP (5) while stimulation of oculomotor parasympathetic provoked the opposite effect (5, 6), which is not mimicked by the application of parasympathetic agonists like physostigmine that evoke a decrease in IOP and increase the humor drainage (7). These observations give support for a role of the autonomic nervous system in the regulation of IOP.

Other physiologic variables are also under the influence of the autonomic nervous system, and, in particular, this influence on cardiovascular variables such as heart rate (HR) and arterial BP has been extensively studied using both invasive and noninvasive methods. A current noninvasive method is a mathematical tool, the fast Fourier transform (FFT). Its application to these cardiovascular variables allows the decomposition of the recorded signal in several harmonics from which a power spectrum is built. The FFT power spectrum of both HR and arterial BP of a human subject, at rest, shows three main bands, which are well correlated with three ranges of frequencies and classified as very low frequencies (VLF; 0-0.03 Hz), low frequencies (LF; 0.03-0.15 Hz), and high frequencies (HF; 0.18-0.4 Hz) (8). The physiologic interpretation of these bands is highly debated but although the meaning of VLF is questionable, evidence exists relating HF to the respiratory rhythm and to the influence of the parasympathetic nervous system (8, 9) while LF band is under the influence of both sympathetic and parasympathetic divisions. Also, the relation LF/HF could reflect the relative influence of the sympathetic/parasympathetic branches of the autonomic nervous system on the recorded variable (10).

Recently, in a study of patients with glaucoma, the application of FFT to a short time recording of IOP of these patients showed the presence of at least four harmonics, where the second, third, and fourth harmonics successfully differentiated between the glaucoma and the normal groups of patients (11).

In the present study, we applied the FFT algorithm to a recording of IOP, in the anesthetized rat, and investigated the possibility of building a power spectrum of this variable to be compared with those of HR and BP obtained from the same animal. A preliminary report of this study has been communicated (12).

METHODS

Surgical and experimental protocol

Experiments were performed in six Wistar rats aged more than 10 weeks (300-350 g) anesthetized with alpha-chloralose (100 mg/Kg, IP) supplemented IV, with the same solution, as necessary. The femoral artery and vein were cannulated for monitoring arterial BP and the administration of drugs and saline, respectively. The trachea was cannulated below the larynx. An adequate level of anesthesia was maintained by ensuring the absence of a withdrawal reflex before the neuromuscular blockade. Rectal temperature was kept at 36.5-38°C by a servo controlled heating blanket (Harvard Apparatus Ltd, UK). The electrocardiogram (ECG) was recorded (Neurolog, Digitimer Ltd) from needle-electrodes inserted into the limbs. HR was derived from ECG (Lectromed, UK). The animal head was then placed in a Kopf sterotaxic frame. In this condition of spontaneous breathing, a period of 5 minutes recording of arterial BP, ECG, and HR was performed. Following the administration of a muscle blocker (pancuronium [4 mg/kg/h]), the animal was artificially ventilated with O_2-enriched air applied using a positive pressure ventilator (Harvard Apparatus Ltd). Artificial ventilation was regulated to maintain end-tidal CO_2 between 4.5 and 5% (ADC Gas Analyser, UK) and its frequency was adjusted to values identical to previous spontaneous ventilation. During this blockade, anesthetic levels were monitored by recordings of BP and HR. An infusion cannula connected to a catheter was introduced in the vitreous body, via pars plana, and IOP recorded connecting the catheter to a pressure transducer (Lectromed, UK). All data were displayed on-line and recorded in videotape with a speed of acquisition of 2 KHz. After stabilization of the preparation, a 5-minute recording of all the variables – arterial BP, ECG, and IOP – was performed.

A third period of 5 minutes recording was taken after two drops of atropine 1%, a vagolytic drug, was instilled in the lower conjunctival fornix.

At the end of the experiment, the animal was killed with an overdose of sodium pentobarbitone (60 mg/kg). All the experimental procedures conformed to the national and EU laws on animal experimentation. The experimental protocol was approved by the Ethical Committee of the Lisbon Faculty of Medicine.

Data analysis

Based on the ECG recording, RR intervals of 5 minutes were measured and depicted in a time series. From the recording of arterial BP, the envelope of systolic BP (SBP) provided the background for the analysis of SBP variability. IOP variability was analyzed from the direct recording of signal provided. In all analyzed variables, DC component was eliminated by subtracting from each value the mean value of each variable. Spectral analysis was performed on stationary records and calculated through the FFT algorithm for a N point succession:

$$H_k = \sum_{0}^{N-1} h_n \, e^{-i\,2\pi\,k\,n}/N$$

considering the signals as discrete temporal series and satisfying the Nyquist theorem:

$$|f|> f_{Nyq} = 1/2\ \Delta t$$

where Δt is the sampling time, to suppress aliasing phenomena. A Hamming window in the time domain

$$w\ (i) = 0.54 + 0.46\ \cos\ (2\pi i/N)$$

was used to attenuate the leakage effect.

As result of the mathematical analysis, a power spectrum density (PSD) was built in which three bands were identified. The limits indicated for these three bands are based on the present observations: a VLF band from 0 to 0.1 Hz and a LF band from 0.1 to 0.6 Hz. Also, a HF band was defined in the range of RF±0.1 Hz, with RF being the basal respiratory frequency (12). The power was calculated as the integral of LF and HF. The normalized power of LF and HF was obtained dividing the integral of each curve by the sum of the integrals of LF and HF (after exclusion of VLF).

The relative potency of sympathetic/parasympathetic was expressed as the relation LF/HF in normalized values. Differences in LF/HF relation for the IOP, RR interval, and SBP before and after atropine were evaluated with the Wilcoxon test and differences were considered significant when $p<0.05$.

RESULTS

In these experiments, recordings were taken under spontaneous and artificial ventilation. In this last case, the rate of artificial ventilation was similar to the basal respiratory frequency before neuromuscular blockade. As described in Methods, HR was analyzed using the RR interval of the ECG and SBP was considered as the superior envelope of the arterial BP recording.

Spontaneous breathing

In spontaneous breathing situation, only the cardiovascular variables – HR (through RR intervals) and SBP – were analyzed. The PSD for both of them showed three bands with the same range of frequencies: VLF 0-0.1 Hz, LF 0.1-0.6 Hz, and HF RF±0.1 Hz (Fig. 1).

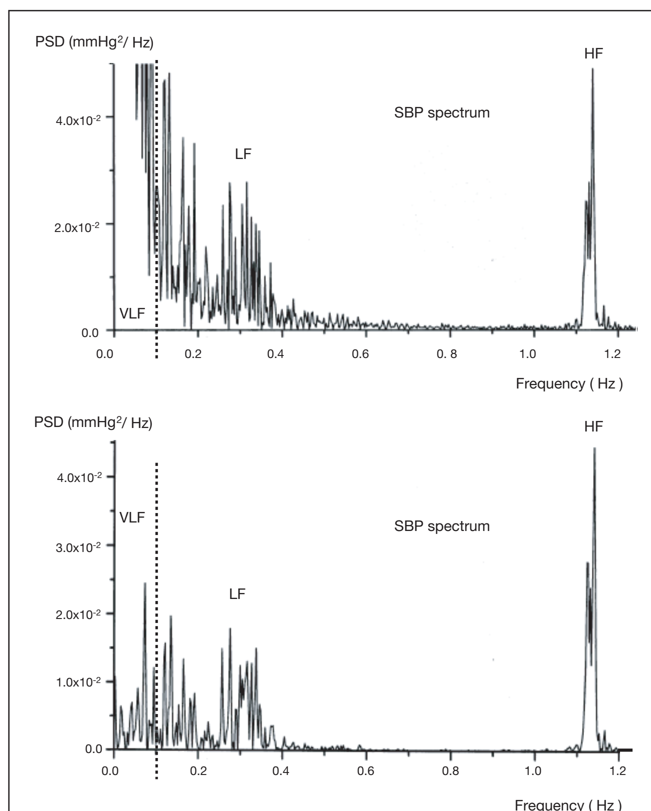

Fig. 1 - *In the upper panel, the power density spectrum of systolic blood pressure under spontaneous ventilation shows three major bands: very low frequency (0-0.1 Hz), low frequency (LF) (0.1-0.6 Hz), and high frequency (HF) (respiratory frequency±0.1 Hz). After removing the DC constant component (lower panel) a clear spectrum is obtained. In this case, LF and HF are not significantly changed.*

The removal of the DC component allowed obtaining a clear spectrum only by decreasing the VLF component, leaving LF and HF unchanged (Fig. 1).

Artificial ventilation

After neuromuscular blockade with pancuronium bromide and under artificial ventilation at the same frequency as spontaneous breathing, the major changes on RR and SBP spectra profile were observed at HF range of frequencies. In fact, HF band became narrower with an increase in its peak value (from the range of 2.0×10^{-2} to the range of 4.0×10^{-2} $mmHg^2/Hz$) when compared with its shape under spontaneous breathing (Fig. 2). In this condition, IOP spectrum showed the presence of three bands in the same range of frequencies of those observed in the RR and SBP spectra. The limits of these three bands are similar in the three spectra with coincidence of the peak of HF as seen in Figure 3.

From normalized values, the relation of LF/HF for RR, SBP, and IOP were, respectively, 0.1±0.03, 0.8±0.40, and 1.3±0.64 (n=6), showing a larger value for IOP than for HR ($p<0.05$) (Fig. 4).

Atropine applied to the conjunctival sac produced a decrease in HF peak value and an increase in the LF/HF relation for IOP (from 1.3±0.64 to 4.4±2.62) and for RR (from 0.1±0.03 to 0.9±0.32) that are significantly higher than those observed before atropine application ($p<0.05$) (Figs. 5 and 6). However, atropine failed to evoke significant changes on SBP-LF/HF relation, which value changed from 0.8±0.40 to 1.0±0.53 ($p=0.043$).

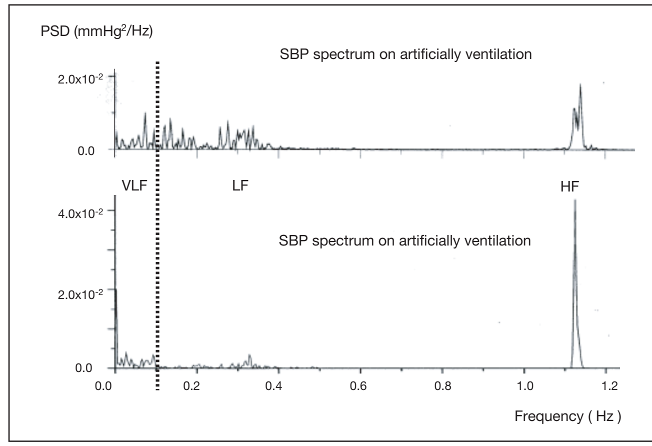

Fig. 2 - *Power density spectrum of systolic blood pressure of an animal breathing spontaneously (top panel) and under artificial ventilation after its treatment with a muscle blocker (lower panel). An increase in high frequency (HF) peak value and a decrease of very low frequency (VLF) and low frequency (LF) bands are observed during artificial ventilation performed at the same rate as the previous spontaneous breathing rate. Please note that the ranges of VLF, LF, and HF are maintained under artificial ventilation.*

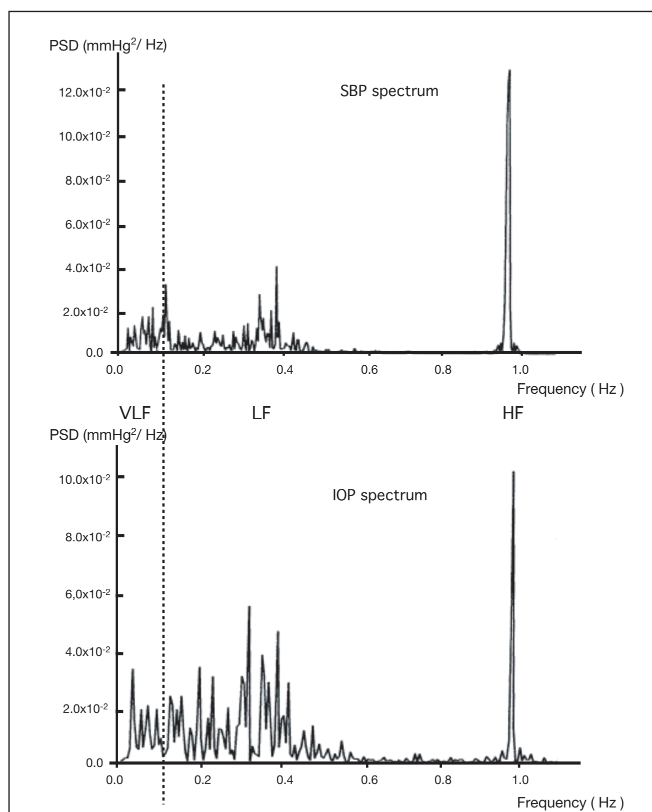

Fig. 3 - *The spectra of the systolic blood pressure and intraocular pressure of the same animal under artificial ventilation. Please note the correlation of bands between the two spectra.*

DISCUSSION

The primary result of our study shows, for the first time, that applying FFT to a recording of IOP, a power spectrum can be built. This spectrum shows three bands that are similar to those which can be observed in the HR and BP spectra obtained using the same mathematical tool. These data give support to the existence of an influence of the autonomic nervous system on IOP.

Studies in human subjects (8-10) have shown that LF and HF bands obtained from HR and BP recordings reflect the autonomic influence on the cardio-

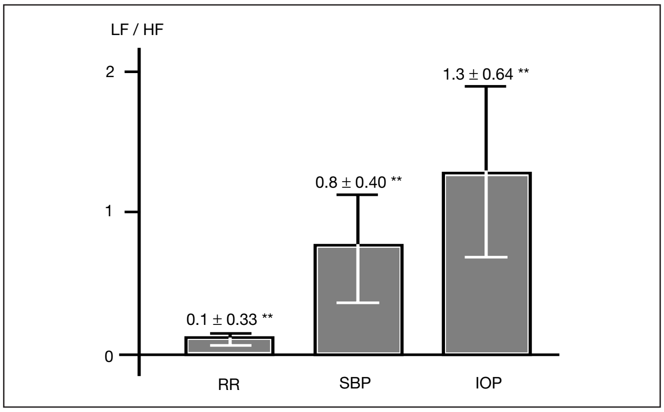

Fig. 4 - *Graph bars showing the low frequency/high frequency relation for each of the three recorded variables under artificial ventilation: RR interval, systolic blood pressure, and intraocular pressure. Data are expressed as mean±SEM, ** statistically significant.*

vascular system. Analyzing the IOP spectrum in the rat, three major bands are seen and we defined its range of frequencies as VLF (0 to 0.1 Hz) and LF (0.1 to 0.6 Hz). HF depends on ventilation and its limits were defined as frequency of ventilation ± 0.1 Hz. These bands are in the same range of frequencies as those observed in the HR and SBP spectra that we defined previously (12), LF being an indicator of sympathetic influence (without excluding a minor parasympathetic interference), HF showing the parasympathetic influence mediated by respiration, and VLF of yet unknown origin. The relation LF/HF reflects the balance between the sympathetic and the parasympathetic outflows (12). The similitude of the three spectra profile (IOP, HR, SBP) strongly suggests that IOP is also affected by the autonomic nervous system. Accounting for this assumption is the fact that the relation LF/HF is higher for IOP when compared with LF/HF relation for HR and, particularly, for SBP revealing a stronger influence of the sympathetic nervous system on this variable. Results also show that atropine application to the eye evoked a dramatic decrease in the peak value of HF band on the IOP spectrum, which supports the parasympathetic origin of this band. The noncomplete abolition of this band could be related either to the fact that the parasympathetic influence in the eye is mediated not only by atropine but also by the vaso-intestinal peptide (VIP) (13) or, alternatively, the observed band could be due to a mechanical influence evoked by the artificial ventilation. Interestingly, atropine, although locally ad-

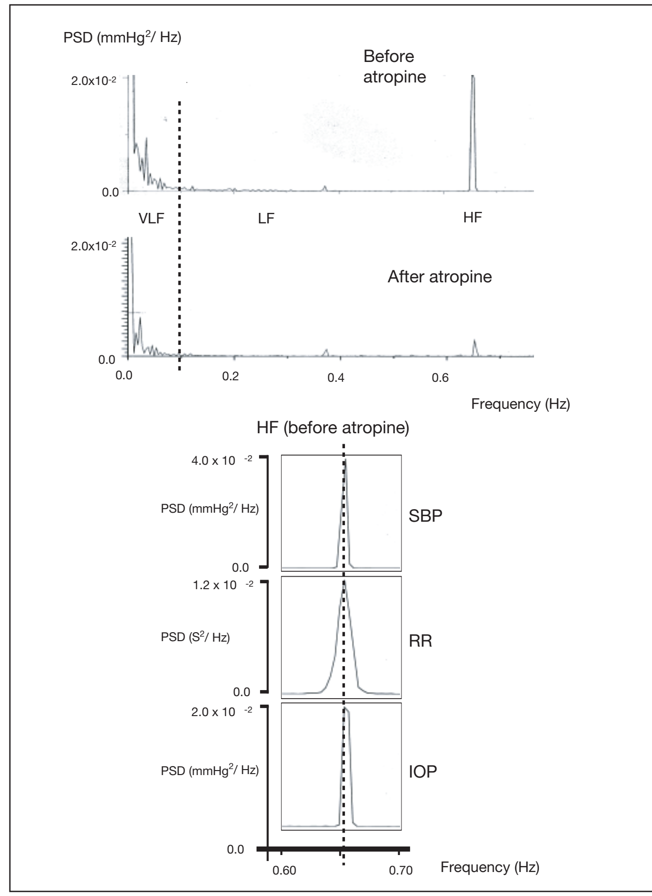

Fig. 5 - *After the instillation of atropine, a vagolytic drug, in the conjunctival sac the decrease in the amplitude of high frequency (HF) band is clearly observed in the intraocular pressure spectrum (upper panel). On the lower panel are shown the HF bands for systolic blood pressure, RR, and intraocular pressure before the administration of atropine in the eye. Note that frequency values for HF are coincident.*

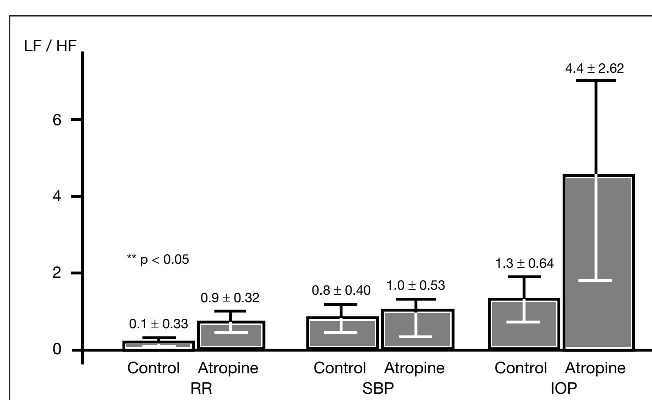

Fig. 6 - *The changes on low frequency/high frequency (LF/HF) relation after atropine application are shown in these graph bars for the three studied variables and compared with LF/HF values obtained before the drug administration (control). Data are expressed as mean±SEM, ** statistically significant.*

ministered, seemed to enter in circulation in a concentration sufficient to influence the heart, which is shown in the modification of LF/HF relation of the RR spectrum.

These novel observations could have important clinical applications as soon as a noninvasive stable recording of IOP is achieved. In fact, the application of this methodology could be used for diagnosis and/or follow-up of patients and/or for the analysis of therapeutic efficacy.

In conclusion, it will allow a simple and lower cost methodology of evaluation of any pathology that affects primary or, as a consequence, the autonomic control of IOP.

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REFERENCES

1. Loewy AD. Autonomic control of the eye. In: Loewy AD, Spyer KM, eds. Central regulation of autonomic functions. Oxford: Oxford University Press, 1990; 269-85.
2. Henkin P, Leitman M, Weitzman E. The diurnal curve in man: new observations. Invest Ophthalmol Vis Sci 1973; 12: 705-7.
3. Bill A. The circulation of the eye. In: Renkin EM, Michel CC, ed. The Microcirculation, Part 2, Handbook of Physiology. Bethesda, MD: American Physiological Society 1984; 1001-35.
4. Braslow RA, Gregory DS. Adrenergic decentralisation modifies the circadian rhythm of interocular pressure. Invest Ophthalmol Vis Sci 1987; 28: 1730-2.
5. Uusitalo R. Effect of sympathetic stimulation on the secretion and outflow of aqueous humor in the rabbit eye. Acta Physiol Scand 1972; 86: 315-26.
6. Macri FJ, Ceravio SJ. Ciliary ganglion stimulation I: effects on aqueous humour inflow and outflow. Invest Ophthalmol Vis Sci 1975; 14: 28-33.
7. Shields MB. Textbook of Glaucoma, ed 2. Baltimore: Williams and Wilkins, 1987; 257-79.
8. Cerutti S, Bianchi AM, Mainardi LT. Spectral analysis of the heart rate variability signal. In: Malik M, Camm AJ, ed. Heart Rate Variability. New York: Futura Publishing Company, 1995; 63-76.
9. Parati G, Saul JP, Di Rienzo M, Mancia M. Spectral analysis of blood pressure and heart rate variability in evaluating cardiovascular regulation. A critical appraisal. Hypertension 1995; 25: 1276-86.
10. Parati G, Di Rienzo M, Omboni S, Mancia G. Computer analysis of blood pressure and heart rate variability in subjects with normal and abnormal autonomic cardiovascular control. In: Mathias CJ, Bannister R, ed. Autonomic failure: a textbook of clinical disorders of the autonomic nervous system, ed 4. Oxford: Oxford University Press, 1999; 211-23.
11. Evans D, Hosking SL, Embleton SL, Morgan AJ, Bartlett JD. Spectral component of the intraocular pressure pulse wave: glaucoma patients versus normal subjects. Graefes Arch Clin Exp Ophthalmol 2002; 240: 475-80.
12. Marques-Neves C, Martins-Baptista A, Rocha I, Delgado E, Boto JP, Silva-Carvalho L. Presence of a power spectrum in variability of intraocular pressure in the anaesthetised rat. CARS 2003; 13: 58.
13. Bill A. Some aspects of ocular circulation. Invest Ophtalmol Vis Sci 1985; 26: 410-24.