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Infrared pupillometry in the assessment of autonomic function

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Abstract

In order to study normal dynamic pupillary function and to determine reference limits for various pupillary variables, 81 healthy subjects aged between 32 and 60 years were examined using a portable infrared pupillometer. Additionally, 36 patients with type I diabetes mellitus were studied. In healthy subjects, sex had no, or only marginal, effect on the responses. Body mass index or smoking habits had no effect on pupillary dynamics. The relative reflex amplitude (RRA) was independent of age. The time to minimum diameter t_{min} was dependent on maximum constriction velocity (MCV) ($P < 0.001$) but not on age, initial diameter or reflex amplitude (RA). The MCV, maximum redilatation velocity (MRV) and time to 75% redilatation ($t_{75\%}$) were strongly dependent on RRA ($P < 0.001$ for each), but age had no or only a marginal effect. No correlation existed between the results of pupillometry and those of Valsalva or deep breathing tests. The lowest normal value for RRA was 29%. The reference limits for MCV, MRV and $t_{75\%}$ were calculated in relation to RRA. The smaller the RRA was, the slower the velocities and the shorter the $t_{75\%}$ were. Using these reference limits, 25% of the diabetic patients without cardiac autonomic neuropathy and 50% with definite cardiac autonomic neuropathy had abnormalities in at least one out of four pupillary variables. It is concluded that infrared pupillometry may be a useful additional method for the assessment of autonomic function.

Keywords: Pupillometry; Autonomic function; Valsalva manoeuvre; Deep breathing test

1. Introduction

During the past 10 years, there have been several reports on the use of pupillometric methods in the assessment of autonomic function, especially in diabetes [1–5] but recently also in other diseases [6,7]. Pupillometry is a valuable method in the examination of autonomic neuropathy because it provides information on the function of cranial autonomic pathways. In combination with standard cardiovascular reflex tests, pupillometry essentially improves the quality of the assessment of the autonomic function of an individual patient. However, there are not too many reports on normal pupillary responses or reference limits suitable for clinical use.

The aim of the present study was to examine dynamic pupillary responses in healthy subjects, to determine reference limits for various pupillary indices and to test the usefulness of the reference limits in a group of diabetic subjects with and without cardiac autonomic neuropathy.

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2. Subjects and methods

The study involved 81 (37 males/44 females) healthy, unmedicated subjects, aged 46 ± 7 years (range 32–60). Their body mass index was 26 ± 4 kg/m^2 (range 18–47). Thirty-seven of them were smokers or ex-smokers. Additionally, 36 (17 males and 19 females) subjects with insulin-dependent diabetes were studied. Their mean age and disease duration was 38 ± 9 years and 20 ± 10 years. According to the standard non-invasive cardiovascular reflex tests (Valsalva manoeuvre, deep breathing test and standing up), 16 of them had definite cardiac autonomic neuropathy (abnormalities in at least two of five heart rate or R-R interval parameters) and 20 had no evidence of cardiac autonomic neuropathy. Of the subjects with cardiac autonomic neuropathy, one had retinopathy. Informed consent was obtained from each subject and the study was approved by the Ethical Committee of the Research Centre.

2.1. Pupillometry

The test was performed using a method described by Levy et al. [5] and the Pupilscan apparatus (Fairville Medical Optics, Amersham, Bucks, UK). Pupillometry was performed after cardiovascular reflex tests. The testing of the left eye was preceded by an adaptation period of about 1 h (including the period taken by cardiovascular reflex testing) in a dim room (about 10 lux). At least ten measurements were performed, four valid tracings were recorded and the curves were averaged. The following indices were calculated: initial diameter (mm), reflex amplitude (RA) (initial diameter – minimum diameter, mm), relative reflex amplitude (RRA) (%), time to minimum diameter (t_{min}) (s), time to 75% redilatation ($t_{75\%}$) (s), maximum constriction velocity (MCV) (mm/s) and maximum redilatation velocity (MRV) (mm/s).

2.2. Valsalva manoeuvre and deep breathing test

Details of the tests have been published elsewhere [8,9]. During the tests, the heart rate and blood pressure were continuously measured. The blood pressure measurements were performed with a non-invasive technique measuring the fast beat-to-beat blood pressure changes (Finapres 2300, Ohmeda, USA) [10]. In the Valsalva manoeuvre the subject maintained a pressure of 40 mmHg for 15 s by blowing through a mouthpiece. The differences between the resting systolic (SBP) and diastolic (DBP) blood pressure (BP) before the strain and the minimum BP during the strain were calculated (SBP/DBP decrease). The magnitude of the rebound hypertension after the strain was calculated as the difference between the resting SBP/DBP and the maximum SBP/DBP during the overshoot (SBP/DBP overshoot). The magnitude of rebound bradycardia was expressed as the Valsalva ratio. The latencies from the end of the strain to the peak of blood pressure overshoot (overshoot time) and from the peak of the blood pressure overshoot to the peak of the rebound bradycardia (bradycardia latency) were calculated [11]. Three valid Valsalva manoeuvres were recorded, the mean of which was used as the final result. In the deep breathing test the subject breathed deeply and continuously at a rate of 6 breaths/min. The E/I ratio was calculated as the ratio at the longest to shortest R-R interval during a respiratory cycle. Six consecutive ratios were averaged.

2.3. Statistical methods

Because of abnormal distribution, natural logarithmic transformation was made for the following variables: t_{min}, $t_{75\%}$ MCV and MRV. The effect of sex was studied by means of covariance analysis with age as a covariate and the effect of smoking habits (smokers + quitters vs. never-smokers) by means of covariance analysis with age and sex as covariates. The effect of age was studied by means of regression analysis and Pearson's correlation analysis. Pearson's partial correlation analysis with age as a covariate was used to study the associations between pupillary variables. Covariance analysis was used to study the independent effects of age, initial diameter, RRA and t_{min} on MCV and MRV. Spearman's partial correlation analysis with age as a covariate was applied to study the correlations between pupillometry and Valsalva and deep breathing tests. The reference values were determined for MCV and MRV by calculating the 90% and 97.5% lower one-sided

Table 1
Effect of sex and age on pupillometry in healthy subjects

Variable	Mean	S.D.	Effect of sex[a]	Effect of age regression equation	P
Initial diameter (mm)	4.93	0.89	ns	8.30–0.07 × age	<0.001
Reflex amplitude (mm)	1.91	0.46	ns	3.63–0.04 × age	<0.001
Relative reflex amplitude (%)	39	6	ns	47.0–0.2 × age	ns
Time to minimum diameter (s)	0.80	0.08	0.03 f>m	0.89–0.002 × age	ns
Time to 75% redilatation (s)	2.07	0.39	0.02 f>m	2.42–0.008 × age	ns
Max constriction velocity (mm/s)	6.83	1.89	ns	13.57–0.15 × age	<0.001
Max redilatation velocity (mm/s)	3.18	1.11	ns	6.00–0.06 × age	<0.001

[a]Covariate: age.

and for $t_{75\%}$ by calculating 90 and 97.5% upper one-sided tolerance limits for individual observation of each variable [12].

3. Results

Table 1 shows the effect of age and sex on the pupillometric variables. Pupillary responses were very similar in females and males, only t_{min} and $t_{75\%}$ were marginally longer in females than in males. Initial diameter, absolute reflex amplitude and velocities decreased significantly with increasing age. The RRA, t_{min} and $t_{75\%}$ were independent of age. Smoking habits or body mass index had no influence on pupillary variables. Pupillary variables did not correlate significantly with blood pressure or heart rate responses in Valsalva manoeuvre or deep breathing test.

Table 2 shows the results of partial correlation analysis between various pupillometric variables. Because MCV and MRV correlated strongly not only with age but also with RRA, the independent effect of these variables on velocities was studied by covariance analysis. The analysis revealed that MCV was very strongly associated with RRA $P < 0.001$, $F = 165.26$), whereas age had no effect ($P = 0.26$, $F = 1.31$). The MRV was strongly associated with RRA ($P < 0.001$, $F = 23.20$), whereas there was only weak association with age ($P = 0.04$, $F = 4.32$). The t_{min} was dependent on MCV ($P < 0.001$, $F = 13.67$) whereas initial diameter or reflex amplitude had no effect on it.

The RRA was independent of age. The 5th, 50th and 95th percentiles for RRA were 29%, 39% and 48%, respectively. The RRA of 29% was regarded as the lowest normal value. Table 3 shows the nor-

Table 2
Correlations between pupillary variables in healthy subjects using Pearson's partial correlation analysis with age as a covariate

	Initial	RRA	t_{min}	$t_{75\%}$	MCV	MRV
Age	−0.55***	−0.22ns	−0.15ns	−0.14ns	−0.52***	−0.42***
Initial		−0.04ns	0.35**	0.39***	0.52***	0.13ns
RRA			0.30**	0.29**	0.66***	0.47***
t_{min}				0.62***	0.22*	−0.11ns
$t_{75\%}$					0.23*	−0.24*
MCV						0.59***

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$; ns, $P > 0.05$.

Table 3
Reference values for maximum constriction and redilatation velocities (mm/s) (MCV and MRV) and time to 75% redilatation(s) ($t_{75\%}$) in relation to relative reflex amplitude (%) (RRA). Expected value (0.50), lower one-sided 90% (0.10) and 97.5% (0.025) tolerance limits for MCV and MRV and expected value (0.50) and upper one-sided 90% (0.90) and 97.5% (0.975) tolerance limits for $t_{75\%}$

	Relative reflex amplitude (%)										
		24	28	32	36	40	44	48	52	56	60
MCV	0.50	4.17	4.72	5.35	6.06	6.86	7.77	8.80	9.97	11.29	12.79
	0.10	3.16	3.59	4.08	4.63	5.25	5.94	6.71	7.79	8.58	9.58
	0.025	2.72	3.10	3.53	4.01	4.54	5.13	5.79	6.54	7.40	8.20
MRV	0.50	2.04	2.27	2.53	2.82	3.14	3.49	3.89	4.33	4.82	5.36
	0.10	1.43	1.60	1.79	2.00	2.22	2.47	2.74	3.05	3.39	3.70
	0.025	1.18	1.32	1.48	1.66	1.85	2.05	2.27	2.52	2.80	3.03
$t_{75\%}$	0.50	1.74	1.82	1.89	1.97	2.06	2.14	2.23	2.33	2.43	2.53
	0.90	2.23	2.32	2.41	2.50	2.61	2.72	2.84	2.97	3.09	3.26
	0.975	2.55	2.64	2.74	2.84	2.96	3.09	3.24	3.38	3.52	3.75

mal values of MCV, MRV and $t_{75\%}$ in relation to RRA.

When the reference values were applied to the diabetic groups, it appeared that in the group without any evidence of cardiovascular autonomic neuropathy, 5 (25%) of the 20 patients had abnormalities in pupillary variables: 1 in RRA, 2 in MCV, 3 in MRV and 2 in $t_{75\%}$. Three of them had abnormalities in two of the variables. As for the diabetic group with definite cardiovascular neuropathy, 8 (50%) of the 16 patients had abnormalities in pupillometry: 2 in RRA, 6 in MCV, 3 in MRV and 3 in $t_{75\%}$. Four of them had abnormalities in at least two of the variables. The subject with retinopathy had normal values in all of the responses.

4. Discussion

Normal reflex responses to light depend on parasympathetic function. The amplitude of the reflex, the time to peak constriction and the maximum velocities of constriction and redilatation reflect parasympathetic events [13]. Studies with adrenoceptor antagonists have indicated that the time to 75% redilatation would reflect peripheral sympathetic function [13], although it has also been reported that there is no evidence of peripheral α-adrenergic activity during redilatation indicating that also redilatation would primarily be transmitted through parasympathetic pathways [5,14].

For the examination of pupillary responses, there is a wide range of techniques available [13]. Pupillometry can be combined to a standard autonomic cardiovascular test battery without essentially lengthening the test session. In our laboratory, the standard cardiovascular test battery with continuous blood pressure measurement with Finapres device takes approx. 45 min and, when pupillometry is combined to it, the total time needed is 1 h. The Pupilscan device used in the present study has the advantage of being simple to use. Pupilscan is also relatively cheap when compared with other devices measuring the pupil function. Intraindividual repeatability has been reported to be acceptable [5]. Pupilscan has, however, also potential limitations [5]. It cannot eliminate the difference in retinal stimulation due to variations in pupil size, the initial pupil diameter is less than dark-adapted pupil size because of the low-intensity cross-illumination and finally, the sampling rate is up to 20 Hz compared with up to 100 Hz with the conventional infrared pupillometers.

Therefore, one must be careful when comparing the results of the Pupilscan device to the results of TV-infrared pupillometry.

The present study revealed that body mass index and smoking habits have no influence on pupillary responses in healthy subjects. Body mass index has been reported to correlate positively with relative reflex amplitude and time to minimum diameter in subjects with diabetes [5]. Also the latency time, which was not measured here, correlates with percentage of body fat [15]. Recently, it has been reported that smokers have attenuated heart rate variability when compared with non-smokers [16,17]. We did not find any published reports on smoking and pupillary responses. The observed lack of correlation between pupillary responses and heart rate and blood pressure responses in the Valsalva manoeuvre and deep breathing tests was an interesting finding, which is in line with the report of Levy et al. [5] on diabetic subjects. They concluded that pupillary responses are only weakly associated with the results of other tests of autonomic function. It should be noted, however, that the correlations between pupillary and cardiovascular responses in diabetic patients were not studied in the present paper.

As for sex differences, most of the pupillary indices were independent of sex. Females had marginally longer t_{min} and $t_{75\%}$ than males but these differences can be regarded as clinically insignificant. The published data on cardiovascular reflex tests indicates some sex differences in autonomic responses [18]. Levy et al. [5] reported no sex differences in the pupillary indices used in the present study. However, because no clinically significant differences in pupillary variables between the sexes seem to exist, the same reference limits can be applied both to males and females.

In determining the reference limits, the dependence of a given variable on age and other variables must be studied. As to the standard autonomic tests based on cardiovascular reflexes, the most important factor is age; thus age-related normal limits are necessary. However, it is reported that dynamic pupillary responses (MCV, MRV, $t_{75\%}$) are mainly dependent on the reflex amplitude while age has only little direct influence [13]. The covariance analysis of the present study is in agreement with this concept: MCV, MRV and $t_{75\%}$ mainly depend on reflex amplitude (or relative reflex amplitude), not on age. Therefore, the reference analysis was performed in relation to reflex amplitude. Relative reflex amplitude was used because it was independent of age, in accordance with Levy et al. [5], whereas absolute reflex amplitude was strongly correlated with age. Because the RRA is independent of sex and age, the same lowest normal limit (29%) can be used for males and females of different age. Also Smith [13] has determined the reference limits of $t_{75\%}$ in relation to reflex amplitude, although she used absolute reflex amplitude. As for MCV and MRV, no reference values related to reflex amplitude have been previously published.

The determined reference limits for RRA, MCV, MRV and $t_{75\%}$ were applied to two groups of diabetic patients, one with and the other without cardiac autonomic neuropathy. The results showed that as much as 25% of the patients without any abnormalities in standard cardiovascular reflex tests had abnormalities in at least one of the pupillary responses. This indicates that pupillometry may be a sensitive method in detecting an early parasympathetic damage. This is not in agreement with the view that central nervous system autonomic pathways are less frequently affected than peripheral ones [5]. However, it should be emphasized that the previous studies were not based on reference values related to the reflex amplitude. It is probable that the reference limits presented here allow more precise and more reliable analysis of the dynamic pupillary responses than the methods used in the previous studies.

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