Pupil Signs of Sympathetic Autonomic Neuropathy in Patients With Type 1 Diabetes

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OBJECTIVE — Pupillary autonomic neuropathy is considered an early sign of the development of systemic autonomic neuropathy. Sympathetic denervation is related to the duration of diabetes and the development of systemic autonomic dysfunction. We investigated pupillary responsiveness to directly and indirectly acting sympathomimetics in type 1 diabetic patients with and without long-term complications, defined as cardiac autonomic neuropathy (CAN), peripheral sensorimotor neuropathy, retinopathy, and nephropathy, and in healthy subjects.

RESEARCH DESIGN AND METHODS — A total of 47 randomly chosen type 1 diabetic patients and 20 healthy subjects were selected for this study. Patients were divided into groups determined by whether they had long-term diabetic complications. Pharmacological tests were performed with cocaine 4%, epinephrine 1%, and pholedrine 5% eye drops. Horizontal pupil diameter (HPD) was measured at the beginning of the pharmacological tests and at defined time points after instillation of the eye drops.

RESULTS — Statistical analysis showed a significantly smaller HPD in the patients before instilling eye drops ($P = 0.011$). In particular, the HPD was significantly smaller in the patient group without CAN when compared with healthy subjects ($P = 0.004$). Maximal cocaine reaction was diminished in the complication group ($P < 0.001$). Epinephrine test, visual acuity, ocular pressure, and HbA$_1c$ did not differ in patients with or without long-term complications. The noncomplication group showed no significant differences in pupillary responses as compared with healthy subjects. The complication group showed a smaller HPD ($P = 0.022$), reduced pupillary responses in the cocaine ($P = 0.037$) and pholedrine tests ($P < 0.001$), and anisocor pupil sizes after instillation of the eye drops ($P = 0.034$).

CONCLUSIONS — Our results clearly show that sympathetic denervation does exist in the pupil of diabetic patients and that it can be rapidly assessed using the cocaine test. These data and the results of the epinephrine test suggest a mixed pre- and postganglionic dysfunction of the sympathetic plexus. The significant smaller HPD in patients without CAN compared with that of healthy subjects could be a sign for early involvement of the pupil function before cardiac manifestation of systemic autonomic diabetic neuropathy.

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Abbreviations: CAN, cardiac autonomic neuropathy; DCCT, Diabetes Control and Complications Trial; HPD, horizontal pupil diameter; MCR, maximum cocaine reaction; MPR, maximum pholedrine reaction; ROC, receiver-operating characteristic; rHPD, reduced horizontal pupil diameter; rMCR, reduced maximum cocaine reaction; rMPR, reduced maximum pholedrine reaction.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.
results, our present study describes the prevalence of sympathetic pupillary dysfunction in patients with type 1 diabetes and the effects of long-term complications compared with healthy subjects.

RESEARCH DESIGN AND METHODS

Subjects
A total of 47 patients with type 1 diabetes, and 20 age- and sex-matched healthy subjects were recruited for the study. The mean age in the diabetic group was 34.3 ± 7.5 years and in the control group was 30.7 ± 7.5 years. The male-to-female ratio was 0.9 in the diabetic group and 0.7 in the control group. The mean duration of diabetes was 11.7 ± 11.9 years. The mean HbA1c was 8.6 ± 2.9% in the diabetic patients and 5.0 ± 0.3% in the healthy subjects.

The diabetic group was subdivided into groups determined by whether they had long-term complications. Of the patients, 16 had no long-term complications. Of the patients without long-term complications, 15 had long-term complications. Of the patients without long-term complications, 16 patients were assigned to the noncomplications group and 5.0% to the complications group.

Methods

A basic ophthalmological check-up was performed at the beginning of the study. The ophthalmological check-up consisted of visual acuity, slit lamp examination of the anterior eye segment, applanation tonometry, Goldmann perimetry, swinging flashlight test, and indirect ophthalmoscopy.

To evaluate the pupillary function, the dark-adapted initial HPD was investigated first. A cocaine 4% test was performed and the HPD was measured 10, 20, and 30 min after instillation of the eye drops. Patients and probands then received an epinephrine 1% test and a pholedrine 5% test. HPD measurements were made in the epinephrine test 10, 20, and 30 min after instillation of the eye drops. When performing the pholedrine test, the HPD was measured 45 min after instillation of the eye drops. The maximum cocaine reaction (MCR) and maximum pholedrine reaction (MPR) were calculated by finding the difference between the HPD at the end of the pupil function tests minus the HPD from the beginning of these tests. The pupil measurements were performed using a Goldmann perimeter and a 15-watt bulb with short indirect exposure while diabetic patients and healthy subjects were in a completely darkened examination room. Each pharmacological pupillary function test was performed on a separate day with an interval of at least 2 days in between to prevent influence of the previously administered eye drops.

Cocaine 4% eye drops inhibit the re-uptake of norepinephrine in the presynaptic neuron and thus allow a longer contact with the postsynaptic receptors. Pre- and postganglionic lesions of the nerve fibers attenuate this pupillary reaction (13,14).

Epinephrine 1% eye drops were used to indirectly show a loss of innervation of the muscle dilator pupillae in the state of postganglionic alteration through hypersensitivity of this iris muscle (14).

Pholedrine 5% eye drops act by stimulating the transmitter release from presynaptic vesicles of the third neuron of the pupillary sympathetic efferent. This effect is lost in altered nerve function because of a decreased number of vesicles and possibly postsympathetic receptors (13,15). Thus, this test directly shows reduced pupillary dilatation with existing postganglionic lesions. Possible parasympathetic nerve lesions do not interfere with the pupillary response caused by sympathomimetic drugs (14).

In addition, the following sets of measurements were performed to evaluate the status of diabetic long-term complications:

- Heart rate variability was determined using an electrocardiograph attached to an oscilloscope with computerized analysis (Cardiette excel 103), yielding the variation coefficient of heart rate variability, mean circular resulance, spectral analysis of the low, middle, and high frequency waves and maximum-to-minimum 30:15 ratio. More than two pathological findings of the six measured values defined CAN (16).
- The drop of systolic blood pressure after standing was taken. A drop of 30 mmHg was defined as abnormal (16).
- 24-h urinary albumin excretion and 24-h creatinine serum–to–urinary ratio.
- Vibration sensation threshold and thermal sensory perception threshold on lower limbs were estimated using a biothesiometer with computerized analysis (Medoc) together with the clinical examination of the muscle proprioceptive reflexes of the upper and lower limb (17).
- HbA1c determination.

The study was approved by the ethical committee of the Otto von Guericke University of Magdeburg, and patients, as well as healthy control subjects, gave their written consent before examination.

Statistical analysis

The results of the pharmacological pupillary function tests were matched to age. Investigation of the right or the left eye showed no significant difference. We used the SPSS computer program for statistical analysis. Arithmetic mean values, medians, SDs, and quartiles were investigated. Statistical tests were performed using the general factorial ANOVA. Using receiver-operating characteristic (ROC) analysis as a standard approach to evaluate the sensitivity and specificity of diagnostic procedures, cut-off points were determined for HPD, MCR, and MPR to estimate the relative risk (RR) for a reduced pupil diameter due to long-term diabetic complications. For comparison of the observed with the expected results,
the χ² test was performed and RR was calculated using the Fisher’s exact test. P < 0.05 was regarded as significant.

RESULTS — The duration of diabetes was significantly different between the noncomplications and the complications group (P = 0.008). The patients in the complications group were significantly older than the patients in the noncomplications group (P < 0.001). There was no significant difference in mean HbA₁c between these two groups.

Initial HPD and absolute values of pupillary responses to cocaine 4% eye drops and MCR are illustrated in Fig. 1. Initial HPD showed significant differences between the noncomplications group (3.77 ± 0.84 mm) and the complications group (3.24 ± 0.67 mm; P = 0.022). Similar data were yielded for the comparison of 20-min pupil diameter between the noncomplications and the complications group (6.06 ± 0.83 vs. 5.38 ± 1.31 mm; P = 0.038). Likewise, data were similar for cocaine 4% eye drops 30 min after instillation (6.88 ± 0.83 vs. 5.31 ± 1.26 mm) (P < 0.001). MCR was significantly diminished in the complications group compared with the noncomplications group (2.29 ± 0.95 vs. 3.13 ± 0.53 mm) (P < 0.001).

The mean pupil diameter, 45 min after instilling pholedrine 5% eye drops, was 6.95 ± 0.87 mm in healthy subjects, 6.81 ± 0.83 mm in diabetic patients without long-term complications, and 5.08 ± 1.20 mm in diabetic patients with long-term complications. MPR showed a mean value of 3.10 ± 0.83 mm in probands, 2.88 ± 1.12 mm in the noncomplications group, and 1.98 ± 0.93 mm in the complications group. Mean values showed significant differences in pupil diameter 45 min after instillation of pholedrine 5% eye drops (P < 0.001) and MPR (P < 0.001) when comparing the noncomplications with the complications group.

Initial HPD and pupillary responses to the mydriatics of diabetic patients without long-term complications showed no significant differences when compared with healthy subjects. Epinephrine 1% did not yield any significant difference between the complications and noncomplications group or compared with probands. When all diabetic patients were compared with probands, HPD was significantly smaller (3.87 ± 0.16 vs. 3.47 ± 0.25 mm; P = 0.011).

Absolute values for the investigated pharmacological pupil function tests are not described in the literature. In most cases, lesions of the sympathetic plexus arising from the spinociliar center were described as unilateral alterations related to local pathological processes; thus, test results are described as relative values compared with the normal eye and pupil function. This assumption could not be applied in this study, since lesions of the sympathetic plexus in both eyes of one individual can be caused by the systemic disease of diabetes. To estimate the RR of the incidence of pathological findings in the cocaine and pholedrine test, in conjunction with long-term diabetic complications, the maximum reaction values were classified into groups with normal and reduced differences between the start and the end of the pupil function test. A reduced MCR (rMCR) and MPR (rMPR) was assumed with pupil diameter differences <2.75 mm using the ROC analysis. The RR for the coincidence of long-term complications was 1.714 for rMCR and 2.112 for rMPR. Table 1 shows even higher RRs for each long-term complication studied.

Initial HPD was defined as reduced (rHPD) when it was <3.75 mm. This value was again chosen as a cut-off point to define pathological pupillary width defined by the ROC analysis. The RR of coincidence of a small dark adapted pupil size together with long-term diabetic complications was 2.864. The risk for each long-term complication as defined above is shown in Table 1. Diabetic patients without any long-term complication had no significantly elevated RR of coincidence of rHPD, as opposed to the probands with RR 1.560 (95% CI 0.773–3.147).

Diabetic patients with long-term complications demonstrated anisocoria pu-

Table 1

<table>
<thead>
<tr>
<th>Long-term complications in general</th>
<th>rHPD</th>
<th>rMCR</th>
<th>rMPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAN</td>
<td>2.864</td>
<td>1.799</td>
<td>2.128</td>
</tr>
<tr>
<td>Peripheral sensomotor neuropathy</td>
<td>1.591</td>
<td>4.261</td>
<td>1.738</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>2.227</td>
<td>3.598</td>
<td>1.966</td>
</tr>
<tr>
<td>Nephropathy</td>
<td>1.803</td>
<td>2.273</td>
<td>3.724</td>
</tr>
<tr>
<td></td>
<td>4.875</td>
<td>6.286</td>
<td>3.339</td>
</tr>
</tbody>
</table>

Data are RR (95% CI).
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Figure 2—Absolute values of HPD at different time points and MCR with SD in millimeters after instillation of cocaine 4% eye drops in healthy subjects (dark gray) versus type 1 diabetic patients without CAN (light gray) and with CAN (white).

Pupil sizes after termination of the pharmacological pupil function tests significantly more frequently than type 1 diabetic patients without long-term alterations (P = 0.034). The RR of coincidence of systemic long-term complications and evidence of anisocoria pupil sizes after testing was 4.074 (1.138–14.581).

To investigate the pupil function as a parameter assessing an alteration of the autonomic nervous system in relation to presence or absence of signs of CAN, we then subdivided the patients with diabetes into two groups: one with CAN and one without (Fig. 2).

Of the patients with diabetes, 19 had no signs of CAN and 28 patients showed evidence of this long-term complication. The mean age was 30.6 ± 6.2 years in the first group and 39.8 ± 8.3 years in the second (P < 0.001). The two groups showed no significant differences when studying the duration of diabetes, with 9.5 ± 10.4 years in the first and 14.9 ± 13.4 years in the second group. HbA1c levels also were not significantly different, with 9.0 ± 3.3% in the group with CAN and 8.3 ± 2.5% in the group without.

In contrast with the results shown above, the diabetic patients without CAN had a significantly smaller initial HPD of 3.43 ± 0.70 mm compared with 3.98 ± 0.61 mm in healthy subjects (P = 0.004). The other parameters of the cocaine and pholedrine test did not yield significant differences. Furthermore, the RR of coincidence of rHPD for diabetic patients without CAN was 2.127 (1.259–3.595) as compared with healthy subjects (P = 0.003).

In the diabetic group without CAN, compared with those with CAN, significant differences were shown for the pupil size 30 min after instillation of cocaine eye drops with 6.48 ± 0.91 and 5.39 ± 1.47 mm (P = 0.008), respectively. For MCR, those without CAN had 2.96 ± 0.54 mm and those with CAN had 2.00 ± 1.05 mm (P = 0.001). The mean pupil diameter 45 min after instilling pholedrine 5% eye drops was 6.04 ± 0.80 mm in diabetic patients without CAN and 5.13 ± 0.48 mm in diabetic patients with CAN (P = 0.024). MPR showed a mean value of 2.50 ± 1.00 mm in the group without CAN and 1.97 ± 1.11 mm in the group with CAN (NS).

In particular, diabetic patients with normal ranges in high-frequency waves of heart spectral analysis as a marker for lesions of the cardiac parasympathetic nerve fibers had a smaller initial HPD (3.65 ± 0.42 mm) than patients with pathological high-frequency wave values (4.07 ± 0.61 mm) (P = 0.030). On the other hand, patients with pathological values for this parameter had a significantly reduced MCR than patients with normal values (1.68 ± 0.63 vs. 2.62 ± 0.42 mm, respectively; P < 0.001). Variation coefficient of heart rate variability showed similar results of initial HPD between patients with normal versus pathological coefficients of variation (3.45 ± 0.44 vs. 3.86 ± 0.45 mm; P = 0.012). MCR was significantly reduced in patients with pathological variation coefficient (2.30 ± 0.44 mm) than in patients with normal ranges (2.64 ± 0.44 mm; P = 0.036). MPR showed significant differences: 1.23 ± 1.35 mm in patients with pathological high frequency waves versus 2.35 ± 0.93 mm in patients with normal values for this cardiac test (P = 0.014).

No significant differences were found when comparing pupillary measurements with low-frequency waves in heart rate variability, as a marker for cardiac sympathetic nerve lesions.

Of the patients without CAN, 28.6% had a peripheral sensomotor neuropathy versus 89.5% of patients with CAN. Of the first group, 21.4% patients had a retinopathy versus 77.8% of the second group, and 7.7% of the first group had a nephropathy versus 38.5% in the diabetic patients without cardiovascular changes. Of the patients without CAN, 50% showed no sign of any other long-term complications.

Ocular pressure and visual acuity showed no significant differences in the investigated groups. Pupillary reaction was independent from the degree of diabetic retinopathy. Patients with proliferative retinopathy had a corrected visual acuity of 0.63 ± 0.34 to standard but without significant values compared with patients with nonproliferative and without retinopathy. In indirect ophthalmoscopy, these patients showed proliferation signs in the periphery of the retina without clear signs of a maculopathy. Moreover, lesions of the afferent pupillomotor nerves belonging to the maculopapillary bunch of the optic nerve were excluded by means of a swinging flashlight test during the ophthalmological check up at the beginning of the examination.

CONCLUSIONS — This study shows that abnormal pupillary responses to pharmacological pupil function tests are evident and thus demonstrate a coincidence with signs of other long-term complications of diabetes. This finding is in accordance with other studies, demonstrating increased pupil light reflex latencies in diabetic patients suffering from CAN (18). Furthermore, significant cor-
relations to duration of diabetes and age were detected (19). Patients with type 1 diabetes with no long-term complications also had normal pupillary responses in the tests mentioned above.

A significantly smaller initial HPD at the beginning of the tests was seen in the diabetes group without CAN when compared with healthy subjects and is regarded as an early sign of involvement of the autonomic nerve system (20). These findings have been corroborated in other studies (12, 21). The correlation with other long-term complications could also explain the small HPD without the evidence of CAN, but may also represent a sign for early involvement of the pupil function before cardiac manifestation of systemic autonomic diabetic neuropathy.

The absence of an association between a small initial pupil size and CAN may be explained by the fact that alterations of the autonomic nerve system can be very nonhomogenous. The evidence of an oval pupil (22) as well as local segment spasms (23) in the iris muscle tissue support this assumption. The iris muscles and nerves of the sympathetic plexus may not be involved to the same degree at the same time. Another explanation is the simultaneous presence of a parasympathetic neuropathy of the eye (24). A small initial pupil diameter can be measured only if parasympathetic function is present.

With increasing duration of diabetes, more nerve fibers are involved, which is evidenced by the strong correlation of the parameters of the cocaine and pholedrine test with systemic long-term complications and correlation of these pupillary function tests with specific cardiac tests. The epinephrine test did not yield significant results, though philedrine reaction was significantly reduced in patients with long-term complications as a sign of involvement of postganglionic sympathetic nerve fibers. Thus, a mixed pre- and post-ganglionic lesion of the ocular sympathetic plexus must be assumed.

The larger dark-adapted initial pupil size in patients with pathological findings in the spectral analysis of the heart rate variability, in combination with reduced maximal cocaine and pholedrine reaction, supports this notion of a presence of a mixed sympathetic and parasympathetic lesion of the ocular nerve plexus with loss of a small dark-adapted pupil size. A small dark-adapted pupil size may be interpreted as an early sign for involvement of the sympathetic pupil function (14), apart from iridic parasympathetic nerve fiber affection before cardiac manifestation of systemic autonomic neuropathy. This inverse correlation between cardiovascular autonomic score and pupil diameter has been shown in previous studies (25).

In the early stages, neuropathy is reversible through improved glycemic control (8). The DCCT data clearly support the benefit of intensive therapy in preventing the appearance of clinical neuropathy (2, 26). Thus, screening for autonomic dysfunction is certainly mandatory in order to prevent its sequelae; studying pupil tests may be one way of diagnosing this condition as early as possible.

References
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