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Neural influences on the iris of diabetic rats and effect of oculomotor nerve crush

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Abstract

Using an animal model where the pupil diameter of the eye in anaesthetized and dark-adapted rats serves as a parameter of autonomic function, we studied the functional recovery of the parasympathetic nerve fibres in the oculomotor nerve after a crush lesion in rats with streptozotocin-induced diabetes compared with normal controls. Prior to the crush lesion, diabetic rats develop significantly ($P < 0.001$) smaller pupils compared with controls, and this occurs early in the course of the diabetes mellitus. As the difference in pupil diameter between control and diabetic rats persists immediately after the crush lesion, when the nervous control of the pupil is entirely due to sympathetic nerves, we suggest that the reduction in pupil diameter is due to a sympathetic neuropathy. Furthermore, we show that the functional recovery of the parasympathetic input to the iris after a crush lesion of the oculomotor nerve is not as good in diabetic rats as it is in normal control rats.

Keywords: Autonomic neuropathy; Diabetic neuropathy; Autonomic nerve regeneration; Pupillometry

1. Introduction

In previous experiments, we have demonstrated that rats with streptozotocin-induced diabetes have smaller pupils than controls, and that this is prevented by treatment with an $ACTH_{4-9}$ analogue [6,8]. It is thought that the smaller pupils in diabetic subjects are due to defective function of the sympathetic nerve supply to the iris rather than to iris muscle myopathy or decreased afferent input resulting from retinopathy [2]. Histological evidence of this sympathetic neuropathy is still lacking and only pharmacological evidence is available [5,8]. In diabetic rats we have demonstrated hypersensitivity to phenylephrine, as early as four weeks after they have been made diabetic [8]. To our knowledge, direct evidence of a sympathetic neuropathy in the iris of diabetic subjects is still lacking.

The aim of the present study was to investigate the (spontaneous) functional recovery of the parasympathetic nerve fibres in the oculomotor nerve after a crush lesion in rats with streptozotocin-induced diabetes compared with normal controls.

2. Materials and methods

2.1. Animals

Female adult rats of an inbred Wistar strain (age 13–14 weeks), weighing approx. 230 g at the onset of the experiment, were used. The animals were housed in Makrolon cages on sawdust and on a 12:12 h light/dark cycle, with food and water ad libitum. The rats were randomly divided into two groups. One group of animals ($n = 22$) received a single intravenous injection of streptozotocin (Zanosar^R, Upjohn, Kalamazoo, MI, USA) in a dose of 50 mg/kg total body weight (b.w.). After one week, blood glucose levels were determined by Haemo-Glukotest^R strips (Boehringer-Mannheim, Mannheim, Germany). Rats with a glucose level higher than 14 mmol/l were considered diabetic [3]. These rats received no insulin. A second group ($n = 12$) consisted of weight-matched, untreated control rats. After eight weeks all animals were subjected

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to surgery (see section 2.2). The average weight then was 360 g for the control rats and 240 g for the diabetic rats.

2.2. Surgical technique

All operations were carried out under general anaesthesia (Hypnorm^R, Duphar, Weesp, The Netherlands, containing 10 mg/ml fluanisone and 0.315 mg/ml fentanyl citrate, dose 0.1 ml/100 g b.w., administered i.m.; in addition, midazolam was administered i.p.: 0.5 mg/rat for control rats and 0.25 mg/rat for the diabetic rats). The rats were immobilised in a stereotactic frame. Both eyes were closed with tape to protect the corneas from dehydration. The entire operation was performed under an operating microscope (Zeiss OpMi-1; lens focal distance 200 mm). A frontotemporal incision was made on the left side, and the temporal muscle was dissected from the temporal bone and reflected. The pars squamosa of the temporal bone was removed up to the floor of the middle fossa with a high-speed air drill. The dura mater was incised along the inferior margin of the craniectomy and the piriform lobe was gently retracted, which was facilitated by the removal of cerebrospinal fluid. Invariably, a small tentorial artery runs at a right angle to the edge of the tentorium. At this level, the oculomotor nerve is covered by a duplication of the dural sheath of the tentorium, which was opened. The oculomotor nerve was easily identified at the skull base, medial to the trigeminal nerve and proximal to the cavernous sinus. A short segment of the nerve was isolated and crushed for 5 s using a modified aneurysm clip. Before the nerve was crushed, the pupil was small, immediately after it became maximally dilated. The crushed nerve was easily repositioned in its original place. The dura mater overlying the piriform lobe was not closed. The temporal muscle was approximated with absorbable Vicryl^R 4-0 sutures (Ethicon, Johnson and Johnson, Amersfoort, The Netherlands), as was the skin. The left eye was protected with chloramphenicol eye ointment.

2.3. Pupil diameter measurements

All measurements were carried out on dark-adapted rats under general anaesthesia (Hypnorm^R, dose 0.1 ml/rat, administered i.m.). The rats were placed under the microscope (Zeiss OpMi-1; magnification ratio ×16), in a darkroom. The microscope front lens was covered with a red filter (590 nm). A 35-mm reflex camera (Canon EOS-650) with electronically-controlled automatic exposure was side-mounted to the operating microscope. The left eye of each rat was brought into focus in a plane parallel with the microscope lens. Using the camera self-timer, the shutter was released after 10 s. Kodak Tmax P3200 professional black-and-white film was used. Before all measurements were made, the focal distance of the microscope lens was tested by taking a photograph of calibrated millimeter paper. All pupil measurements were based on this calibration. The baseline measurements were performed before the animals were randomised into two groups. A photograph was taken after 4 and 8 weeks and directly after the crush lesion. Thereafter, all animals were photographed at 2-day intervals. After developing the exposed films, all slides were mounted and projected onto a white screen for measurement of pupil diameter. All slides were measured independently by two investigators on separate occasions (limits of agreement −0.06 to +0.06 mm) [1].

2.4. Data analysis

Multivariate analysis of variance (MANOVA) was used to analyse all the pupil diameter measurements followed by a Student's t-test, using the Statistical Package for the Social Sciences (SPSS) software. Data obtained from rats that died during the course of the experiment were excluded. The cause of death was usually suffocation during anaesthesia for the pupil diameter measurements.

3. Results

22 rats in the diabetic group and 12 rats in the control group were used for final analysis. Streptozotocin-treated rats rapidly developed a high blood glucose level which, after one week, was 20–47 mmol/l.

At the onset of the experiment the mean diameter of the contracted pupil was 0.56 mm (SE 0.02) for the diabetic group and 0.56 mm (SE 0.02) for the control group (t test, $t = 0.33$, df = 32, $P < 0.75$). In the course of 8 weeks the pupil diameter in the control group increased to 0.68 mm (SE 0.04), whilst in the diabetic group the pupil diameter decreased to 0.48 mm (SE 0.02)(MANOVA, $F_{1,29} = 15.58$, $P < 0.001$). This difference in pupil diameter was already

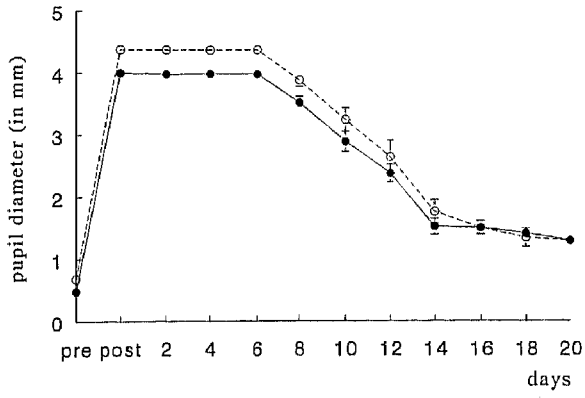

Fig. 1. Pupil diameter (mean with SE) after crushing the oculomotor nerve intracranially in control rats (○; $n = 12$, open circles), and diabetic rats (●; $n = 22$, filled circles). Spontaneous recovery occurs in approx. 16 days. The functional recovery of the parasympathetic input to the iris is less effective in diabetic rats than in normal control rats. (pre, pre-operative; post, post-operative).

statistically significant after 4 weeks (t test, $t = -2.74$, df = 30, $P < 0.01$).

After the crush lesion of the oculomotor nerve the mean pupil diameter increased to 4.00 mm (SE 0.06) in the diabetic group and to 4.38 mm (SE 0.07) in the control group t-test, $t = -4.19$, df = 25, $P < .001$), and remained virtually unchanged in both groups for 6 days (Fig. 1).

Six days after the nerve crush lesion the pupils in both groups started to become smaller, and in the course of the next eight days the mean pupil diameter decreased to 1.51 mm (SE 0.14) in the diabetic group and to 1.75 mm (SE 0.2) in the control group (t-test day 14, $t = 1.01$, df = 19, $P < 0.32$). Thereafter the pupil diameter only slightly decreased to 1.27 mm (SE 0.09) in the diabetic group and to 1.27 mm (SE 0.1) also in the control group (t test day 20, $t = 0.01$, df = 19, $P < .99$). Compared to the pupil diameter immediately prior to the crush lesion, the mean pupil diameter in the control rats remained 1.8-times greater, whereas the mean pupil diameter in the diabetic rats remained 2.6-times greater.

4. Discussion

The present study confirms that streptozotocin-induced diabetic rats develop significantly smaller pupils compared with controls, and that this occurs early in the course of the diabetes mellitus. Furthermore, we show that the functional recovery of the parasympathetic input to the iris after a crush lesion of the oculomotor nerve is not as good in diabetic rats as in normal controls.

We previously presented pharmacological evidence suggesting that the smaller pupils in diabetic rats are caused by a sympathetic neuropathy, using the concept of denervation hypersensitivity to phenylephrine eyedrops [8]. With the animal model developed for this study we were able to show that a reduction in pupil diameter occurs in diabetic conditions in rats as in humans. We can not exclude the possibility, however, that the pupils of diabetic rats are affected by the anaesthetic to a greater extent than the controls.

After a crush lesion of the oculomotor nerve, complete degeneration of the nerve distal to the lesion occurs. When the parasympathetic input to the iris is thus abolished, the nervous control of the pupil immediately after the crush is entirely due to sympathetic nerves. In these conditions, the difference in pupil diameter between control and diabetic rats persists. It can, therefore, be suggested that the reduced pupil diameter in the diabetic rat is due to a reduced effect of sympathetic nerves. This hypothesis is in agreement with our earlier suggestion that the reduction in pupil diameter is due to a sympathetic neuropathy [8].

The mean pupil diameter in normal control rats 20 days after the crush lesion of the oculomotor nerve was 1.8-times greater than the pupil diameter immediately prior to the crush. The extent of this functional recovery in normal rats is comparable to that found in our earlier experiments [7], and to that found by Fernandez et al. [4]. The mean pupil diameter in the diabetic rats 20 days after the crush lesion of the oculomotor nerve, was 2.6-times greater than the pupil diameter immediately prior to the crush, implying poor recovery in the diabetic rats when compared to normal controls. As the difference in pupil diameter between the diabetic and the control rats was no longer present after 14 days, the sympathetic nerves, even in the presence of a sympathetic neuropathy, still appear to be able to counteract the action of the parasympathetic nerve fibres. This again implies that the functional recovery of the parasympathetic input to the iris is less effective in diabetic rats than in normal control rats. However, our model does not differentiate between a sympathetic and a parasympathetic autonomic dysfunction, so we do not know whether a parasympathetic dysfunction was already present prior to the crush lesion.

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