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The carotid body of the spontaneous insulin-dependent diabetic rat

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Abstract

The carotid bodies from adult spontaneous insulin-dependent diabetic rats (strain BB/S) were perfusion-fixed at normal arterial blood pressure with 3% phosphate-buffered glutaraldehyde and compared with the organs from control rats (strain BB/Sc) prepared in the same way. Serial 5-µm sections were cut, stained, and using an interactive image analysis system, were analysed to determine the volumes of the carotid body and its vascular and extravascular compartments. There was no evidence of systemic arterial disease in the carotid stem arteries in either group of animals, and the microvasculature of the organs appeared normal by light microscopy. The volume of the carotid body was unchanged 3 months after the onset of diabetes but was increased at 6 months. The total vascular volume of the organ was unchanged, but the volume of the small vessels (5-12 µm) was increased. In the control group the small vessels comprised 5% of the total volume of the carotid body, or about 44% of the vascular compartment. The percentage of small vessels increased at 3 months in the diabetic group, but had returned to normal at 6 months. The extravascular volume followed the same pattern as the total carotid body volume and so did not change appreciably when expressed as a percentage of the total volume of the organ. The increase in size of the carotid body in diabetic rats is due, therefore, to an augmented extravascular volume. In one diabetic specimen the carotid sinus nerve showed signs of diabetic neuropathy, axonal swelling and intramyelinic oedema. The clinical implications of these results are discussed.

Key words
- Diabetic rat
- Carotid body
- Quantitative morphology
- Vascular compartment

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The results are reported here of an investigation of the comparison of the sizes of the various compartments of the carotid body of the spontaneous insulin-dependent diabetic rat with those of the normal organ. The carotid bodies were analysed quantitatively and particular attention was paid to the volume and proportion of the small vessels (5-12-µm in diameter) in the vascular compartment as defined by us together with the density of small vessels. The term "small vessels" has been defined and used previously by us (1).

Carotid bifurcation regions were examined bilaterally in 3 control adult rats of either sex (strain BB/Sc; body weight 301-443 g) and 6 spontaneous insulin-dependent diabetic male rats (strain BB/S; body weight

276-412 g) of the same age (Supplier: Southampton University, Southampton, Hampshire, UK). The latter were investigated in two stages at a nominal 3 months and 6 months after the onset of the disease which commenced at an age of 83.6 ± 8.3 (mean ± SD) days (range 69-95).

Details of the perfusion-fixation technique used by us to fix the carotid bifurcation regions in rats have been reported previously (2). Briefly, the animals were anaesthetized with pentobarbitone sodium, 40 mg/kg, intraperitoneally, and after administering heparin (Monoparin, 1000-1500 IU/kg, *iv*; CP Pharmaceuticals Ltd, Wrexham, Clwyd, UK) to render the blood incoagulable, the chest was opened by a median sternotomy, the animals were quickly bled to death via the right atrium, and perfusion of the carotid bifurcations was immediately begun via the ascending aorta. Perfusion was commenced with sodium chloride (154 mmol/l) at a pressure of 100 mmHg (temperature 37°C) and continued until the effluent from the right atrium was visually clear of blood, and was followed by 3% glutaraldehyde in isotonic phosphate buffer (pH = 7.3) at the same pressure and temperature for 5 min. Each carotid bifurcation region was block dissected and prepared routinely for light microscopy and serial 5-µm sections of paraffin wax blocks of the carotid body were cut and stained using a modification of the Martius Scarlet Blue method for fibrin (1). The procedure for analysis of each section of the carotid body has been described previously in detail (3).

Before anaesthesia and perfusion, it was ensured that the general condition, state of consciousness and neuromuscular coordinations in the control animals were normal. There was no evidence of ketosis. The diabetic animals, on the other hand, showed lethargy, drowsiness and poor neuromuscular coordination. Records of tests for urinary glucose and ketones were kept for the BB/S rats from birth and the date of onset of diabetes was noted when tests became positive for urinary glucose and ketones. Thereafter, the animals were treated with insulin (heat-treated ultralente bovine insulin), the dose being adjusted to prevent glycosuria, ketonuria and other clinical features of diabetes. Prior to perfusion of the carotid bifurcation regions blood glucose levels were 0.5-1.0 mmol/l.

From an analysis of histological sections taken at sample intervals of 50 µm, the following information was obtained by estimations using Simpson's rule: 1) carotid body area and volume; 2) total vascular area and volume; 3) extravascular area and volume by subtraction; 4) small vessel endothelial surface area, i.e., a measurement of the surface area actually based on the external surface of the endothelial cells; 5) large vessel endothelial surface area, and 6) ratio of small vessel endothelial surface area to the carotid body volume.

Where appropriate, group values are reported as means ± SD. The average result for each rat was taken as the unit of measurement in the statistical analysis, as measurements within each rat could not *a priori* be considered independently. The comparisons between the three groups (control, 3 and 6 months of diabetes) were made using one-way analysis of variance. Where this was significant a Sidak test for multiple comparisons was performed to obtain P-values for the comparisons between any two groups. Bartlett's test was used to test for unequal variances in the three groups. STATA (4) was used for all statistical analyses. Values were taken as significant if $P<0.05$. In this initial investigation using small numbers of rats per group, it is impossible to know whether the underlying distributions are homoscedastic or normally distributed. The former is a necessary condition for any test using small numbers and the latter useful for parametric tests with small numbers. Although there was no evidence of heteroscedasticity or non-normality with such small

numbers, the power to detect differences would be very small, and therefore the results should be interpreted with some caution.

The general topographical description of the carotid bodies agreed with that given previously (2). There were no differences in the size and shape of the organ between sexes and the small arterioles entering the caudal pole of the organ ran rostrally in a pattern supplying the capillary beds of type 1 and 2 cells and connective tissue matrix (Figure 1A) (2).

Type 1 and 2 cells appeared normal at a microscopic level, as did the walls of the systemic arteries in the carotid bifurcation regions. In one specimen examined at 6 months after the onset of diabetes, signs of axonal swelling and intramyelinic oedema in the carotid sinus nerve were observed, which is suggestive of diabetic neuropathy. These changes were noted in the nerve as it was traced caudally in the serial sections to approach the rostral pole of the carotid body and enter the organ (Figure 1B). There was no evidence of histopathological changes in the sympathetic nerves approaching or entering the carotid bodies of the diabetic animals.

The mean values for the volume of the carotid bodies in the 3-month stage of the diabetic rats decreased, but not significantly. On the other hand, the volume at the 6-month stage was increased significantly compared to both the controls (P = 0.016) and the 3-month stage (P = 0.003) by 95% and 215%, respectively (Figure 2).

The values for volumes of the total vasculature, small vessel and extravascular compartments of the carotid body in the control group of animals and in the 3-month and 6-month stages in the diabetic animals are shown in Figure 2. Although the total vascular volume was unchanged at 3 and 6 months after the onset of diabetes, the small vessel volume remained unchanged at 3 months, but increased by 107% at 6 months (P = 0.051); the volume at 6 months was also larger than at 3 months by 125% (P = 0.038).

Figure 2 shows a comparison of the values for extravascular volume. Although at 3 months after the onset of diabetes there was no significant change (P = 0.229), at 6 months extravascular volume was increased significantly by 103% (P = 0.008) and was larger

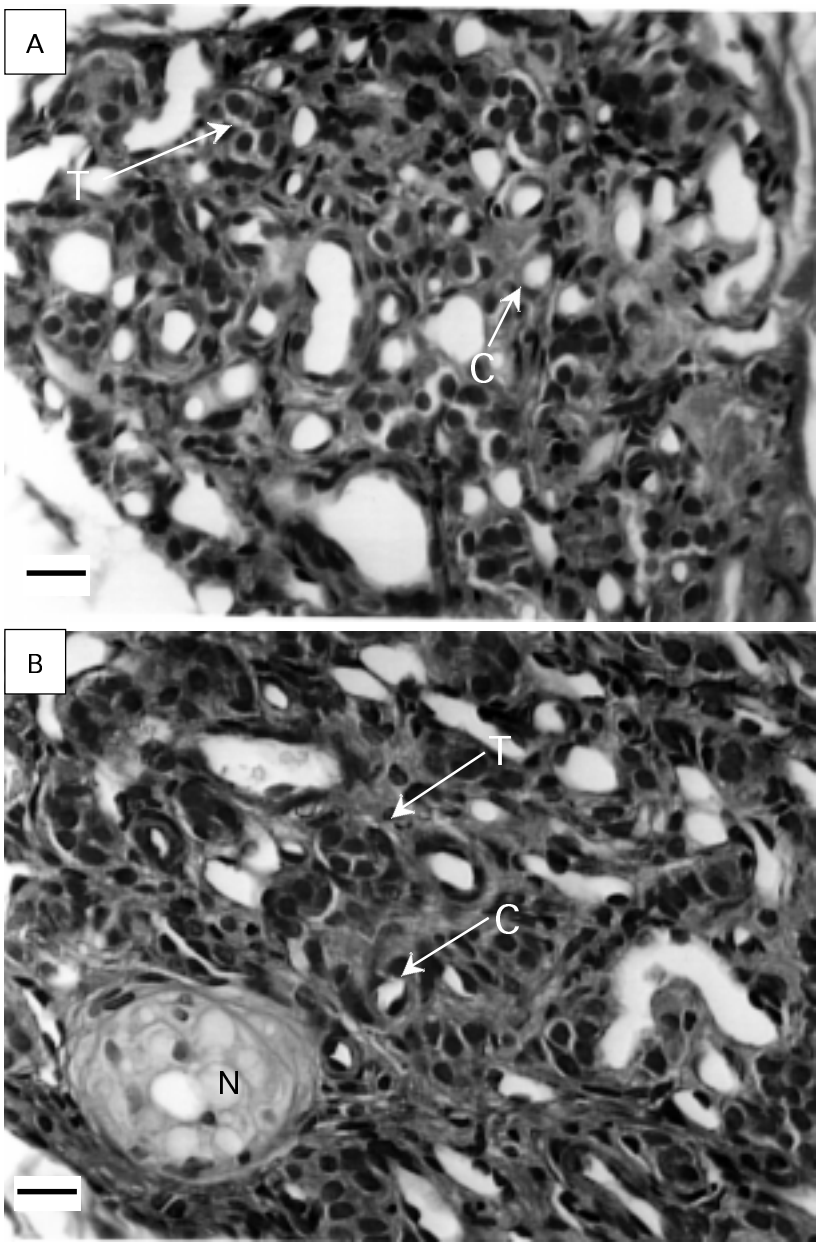

Figure 1 - Photomicrograph of the carotid body from a normal control rat (A) and from a diabetic rat, 141 days from the onset of diabetes (B). Section thickness: 5 µm. MSB stain. Scale bar: 20 µm. Note type 1 cells (T), capillary (C) and axonal swelling of the carotid sinus nerve (N).

than at 3 months by 241% (P = 0.001).

These values for vascular and extravascular volumes are also expressed in Figure 2 as a percentage of the carotid body volume. At the 3-month stage of diabetes, the total vascular volume as a percentage of the carotid body volume increased by 57% (P = 0.091), but at 6 months there was no difference from control (P = 0.84). The value at 6 months was also less than at 3 months (P =

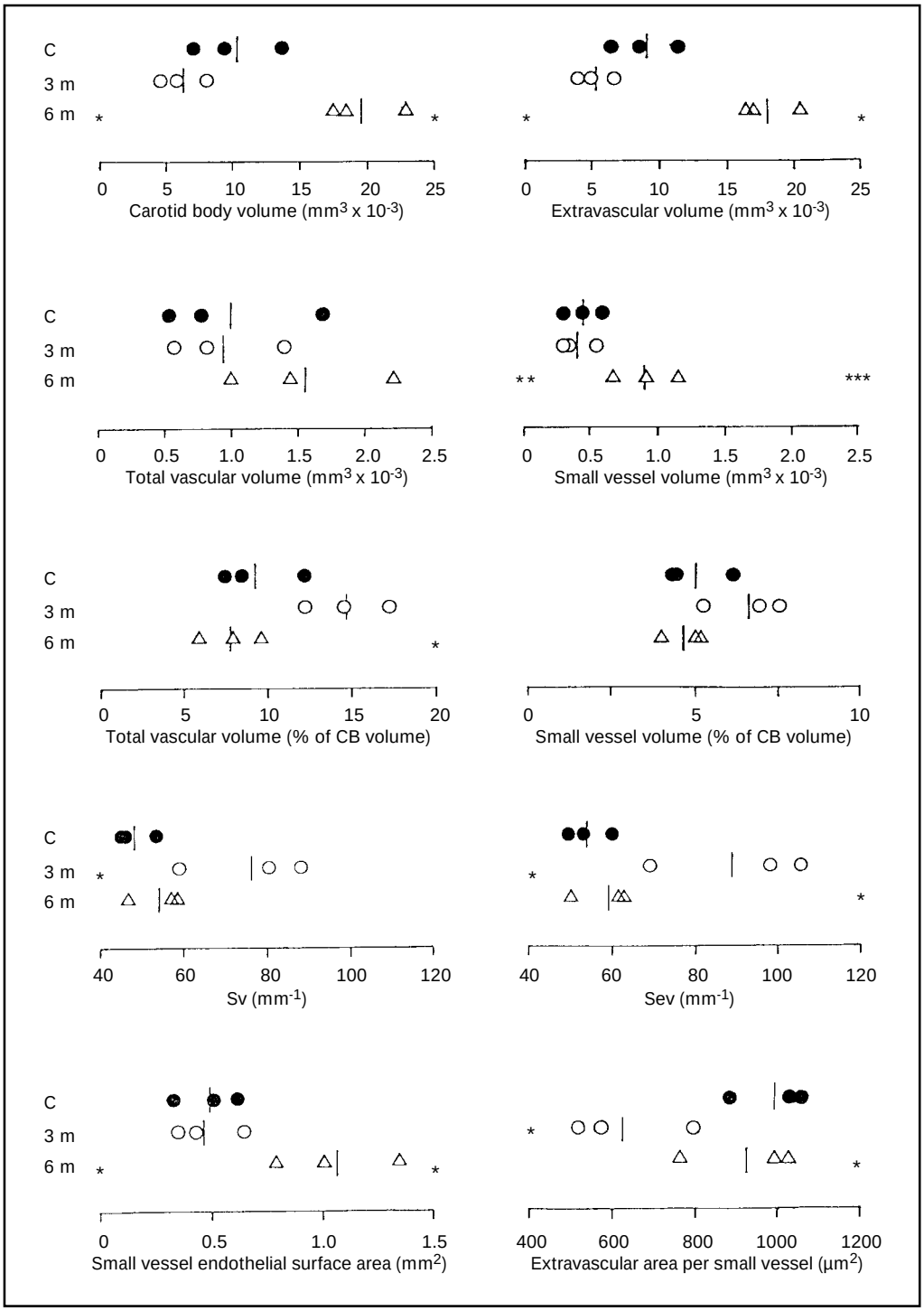

Figure 2 - The relationships between the dimensions of the carotid bodies (CB) in control animals (C, filled circles) and in diabetic animals, 3 months (m) (open circles) and 6 months (open triangles) after the onset of diabetes. Values are the average of the two carotid bodies of each animal. *Vertical lines*, Mean value for each group. Statistical analyses: Asterisks on the left-hand side are shown for a group when it differs significantly from the control group. Asterisks on the right-hand side are shown when the 6-month group is significantly different from the 3-month group. *P<0.05, **P<0.01, ***P<0.001 (all P values after allowing for multiple comparisons). Sv, Small vessel endothelial area per unit carotid body volume; Sev, small vessel endothelial surface area per unit extravascular volume.

0.034). Regarding the small vessel volume per unit carotid body volume, there were no differences between the values for the control group and the two stages of the diabetic groups of animals (Figure 2).

Although the mean small vessel endothelial surface area was unchanged at the 3-month stage in the diabetic animals, it was increased at the 6-month stage ($P = 0.033$), the latter value also being significantly greater than that at 3 months ($P = 0.031$). The values for the small vessel endothelial surface area per unit carotid body volume (Sv) and per unit extravascular volume (Sev) were increased but only at the 3-month stage ($P = 0.039$ and $P = 0.030$, respectively) (Figure 2). The mean extravascular area perfused by each small vessel was reduced at the 3-month stage compared with the control ($P = 0.043$), but recovered to its original level at the 6-month stage. Thus, the value at 6 months was possibly less than that at 3 months ($P = 0.09$; Figure 2). This result reflects the reduction in extravascular volume at the 3-month stage and the combined increases in extravascular and small vessel volumes at the 6-month stage of diabetes, respectively (Figure 2).

These results indicate that in the spontaneous insulin-dependent diabetic rat the volume of the carotid body increased in size particularly in the sixth month after the onset of diabetes. This change was due entirely to an enlargement of the extravascular compartment of the organ, since the total vascular volume was not appreciably affected. Although the small vessel volume and endothelial surface area increased over this period, there was no change in the volume when expressed in proportion to the increased size of the organ. Otherwise, no changes were discernible in the microvasculature of the organ, at least by light microscopy.

Our study is based on relatively small numbers of diabetic rats. In practical terms, however, it is difficult to maintain a large number of BB/S animals with overt diabetes, and the number of animals available to us from our suppliers was correspondingly limited. Because of this and because this was an initial investigation, power calculations were not performed.

Compared with values for the control animals, there was a biphasic change in some of the variables. There was a small fall, followed by a rise above the control value, in the total volume of the organ, but no change in the total vascular volume. This means that the total vascular volume, expressed as a percentage of the total carotid body volume, increased initially and then fell to approximately the same level as the controls. The small vessel volume, again expressed as a percentage of the carotid body volume, remained unchanged. The simplest explanation of these changes is that they are due predominantly to alterations in the volume of the extravascular compartment of the carotid body. This is supported by appropriate measurements indicating that the onset of diabetes is associated with no significant change in extravascular volume 3 months after the onset of diabetes, followed by an increase above the control level at the 6-month stage (Figure 2). Our findings do not enable us to identify the cause of this increase in extracellular volume, that is, whether it is determined by changes in volume of the type 1 and 2 cells and/or by changes in the tissues of the extravascular extracellular compartment of the organ, i.e., collagenous connective tissue, lymphocytes, autonomic nerves and occasional autonomic ganglion cells. In this connection, it was found in patients with diabetes mellitus that whilst the fraction of the carotid body lobules occupied by cells was decreased, that occupied by connective tissue was increased (5).

Previous studies carried out by us indicated that whereas the size of the total vascular volume of the carotid body may differ between species, the small vessel volume, expressed as a percentage of the total carotid

body volume, is remarkably constant in perfusion-fixed material between 4.5 and 7%, for example, in Wistar rats, rats of the WKY/OLA strain, cats and a non-human primate (1,2,3,6). The same is true for the BB/Sc control and BB/S diabetic rats used in the present study. No detailed studies have been carried out, however, in an attempt to explain the differences in the size of the total vascular compartment in different species. A possible explanation is that the vessels at the periphery of the organ constituting the venous plexus are variable in size and are partly included within the well-defined perimeter of connective tissue embracing the type 1 and 2 cells (7).

Regarding the functional significance of our observed structural changes, there are two features which might affect the carotid body neurogenic drive. First, the axonal swelling and intramyelinic oedema in the carotid sinus nerve seen in one of our diabetic specimens are suggestive of diabetic neuropathy. An alternative view is that the intramyelinic oedema is due to an accumulation of typical inclusions in macrophages, but it should be pointed out that, whereas this is a common finding in diabetic rats, macrophages were not observed in this particular study in connection with the carotid sinus nerve. Whichever view is adopted, these histopathological findings alone could, through impairment of nerve conduction, be responsible for the diminished arterial chemoreceptor drive. Second, in the absence of changes in the vasculature of the carotid bodies and in the appearance of the type 1 and 2 cells in our specimens, the hypoxic sensitivity of the organ would be expected to increase on account of the enlargement of the extravascular compartment augmenting the diffusion distance between capillaries and tissue, and hence lowering the carotid body tissue PO_2. The extent of the morphological changes in the carotid body and its nerve supply will determine the net effect of these opposing mechanisms on peripheral chemoreceptor function. Experimental and clinical findings favour the view, however, that in diabetes there is a reduced chemoreceptor drive of neurogenic origin. Thus, the cardio-inhibitory and systemic hypertensive responses occurring after intravenous injections of potassium cyanide, an arterial chemoreceptor stimulant, in streptozotocin-diabetic rats were attenuated compared to the responses in a control group of animals (8).

Furthermore, in diabetic patients, the normal hypoxic hyperventilatory response which is reflexly engendered through stimulation of the carotid bodies (9) is either unaffected (10) or blunted or abolished in some cases (11-13), but the size of the respiratory responses does not seem to be dependent on the duration of the diabetes (13). Peripheral chemosensitivity, as indicated by the hypercapnic hyperventilatory responses, is also reduced in cases of diabetic neuropathy (14). In young diabetics with severe autonomic neuropathy, sudden cardiorespiratory arrest has been reported, which was considered to be primarily of respiratory origin (15). The breath-hold time is lengthened compared with normal subjects (12). These observations suggest that at least in some cases of diabetes the carotid body drive is diminished, or even abolished, possibly through impaired conduction in the carotid sinus nerves.

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