neuroReport

NeuroReport 3, 987–989 (1992)

The influence of haematocrit and blood glucose on cerebral blood flow in normal and in diabetic rats

Gitte M. Knudsen,[CA] Enrico Tedeschi and Johannes Jakobsen

Department of Neurology, University Clinic, Rigshospitalet 9, Blegdamsvej, 2100 Copenhagen Ø, DK-Denmark

[CA] Corresponding Author

CEREBRAL blood flow (CBF) was measured at varying haematocrit values in 8 streptozotocin-diabetic and in 7 control rats using the intracarotid ^{133}Xe technique. A hyperbolic relationship between CBF and haematocrit was established for the individual rats in both groups. Diabetic animals showed a preserved CBF response to changes to haematocrit. In 10 normal rats, CBF was measured during acute hyperglycaemia induced by intraperitoneal glucose injection. A significant, inverse correlation was found between CBF and blood glucose. We conclude that the CBF response to changes in haematocrit and thereby in pO_2 is preserved in experimental diabetes. Secondly, in acute hyperglycaemia CBF varies inversely with blood glucose, by mechanisms not fully understood.

Key words: Cerebral blood flow; Haematocrit; Blood glucose; Streptozotocin diabetes

Introduction

In patients with long-term diabetes, impaired cerebral blood flow (CBF) response to CO_2 has been reported[1,2] and this impairment has been attributed to a decreased CO_2 sensitivity of the cerebral vasculature. The physiological significance of this finding is, however, not clear. Cerebrovascular diseases are more frequent in diabetic patients and are often related to a poor outcome. It was therefore of interest to investigate the CBF responsiveness to changes in blood oxygen delivery capacity, as measured by the CBF reactivity to alterations in blood haematocrit. Elevated haematocrit causes a reduction in CBF[3–5] which seems to be solely mediated through cerebral pO_2 regulatory mechanisms and not by rheology *per se*.

Furthermore, in streptozotocin (STZ) diabetes and in acute hyperglycaemia induced by intraperitoneal glucose injection, CBF decreases, at least in some regions.[6,7] Increased haematocrit has been observed in untreated diabetic rats,[8] and may be responsible for the reduction in CBF. Finally, the relationship between haematocrit (or haemoglobin) and CBF has been studied in dogs[9] and in humans[3] but to our knowledge has not been determined for rats. The present study was carried out in order to assess this relationship in normal rats and to determine whether the normal dependency of CBF on haematocrit is preserved in experimental diabetes. Furthermore, the relationship between blood glucose levels and CBF was established in non-diabetic acutely hyperglycaemic rats.

Materials and Methods

Twenty-five age- and weight-matched male Wistar rats were randomly allocated into three groups: In 8 rats with 3 weeks duration of STZ-diabetes and in 7 control rats, haematocrit was varied, and in 10 normal rats, acute hyperglycaemia was induced by means of i.p. glucose injection. For induction of diabetes, streptozotocin was injected into a tail vein at a dose of 80 mg kg^{-1}. Only rats with a blood glucose value higher than 15 mmol l^{-1} the following day and at the day of the experiment 3 weeks later, were included. Following a 4% halothane-induced anaesthesia, animals were tracheostomized and artificially ventilated with an animal respirator. Halothane was then reduced to 0.7% in a gas mixture of 70% N_2O and 30% O_2, and the rats were immobilized with succinyl choline 20 mg kg^{-1} i.v. The level of anaesthesia was continuously monitored with blood pressure and heart rate recordings.

Skin and muscles of the scalp were deflected and all extracerebral branches from the right external carotid artery and the pterygopalatine artery were ligated in order to avoid the registration of extracranial radiation.[10] A polyethylene catheter for $^{133}Xenon$ injection was placed with the tip at the carotid bifurcation so that flow obstruction of the right internal carotid artery was avoided. Both femoral arteries were cannulated for continuous recording of mean arterial blood pressure and for measurement of blood glucose, haematocrit and blood gases. pH, pCO_2 and pO_2 were measured by an ABL30 acid-base analyser (Radiometer, Copenhagen). A femoral vein was cannulated to enable blood and drug infusion. The animals were heparinized and rectal temperature was kept close to 37°C (± 0.5°C) by means of a heated operation table. All wounds were repeatedly infiltrated with lidocaine 1%. On completion of the surgical preparations, which took approximately 90 min, the animals were allowed to rest for at least 30 min.

For each CBF measurement, a 20 µl bolus of the radioactive, inert gas ^{133}Xe dissolved in saline (3.5 mCi ml^{-1}) was injected into the carotid catheter and the

washout curve was obtained with a collimated NaI (Tl) crystal (aperture 8 mm) placed over the hemisphere ipsilateral to the injection catheter. CBF was calculated from the initial slope of a semilog plot of the ^{133}Xenon washout curves,[11] and was, if necessary, pCO_2 corrected. At least three CBF measurements were performed in order to establish baseline CBF values. Since changes in haematocrit alter the value of the partition coefficient (lambda) for Xenon, CBF was corrected for these changes according to Chen *et al.*[12]

In the diabetic and the control groups, haematocrit was increased by infusion of 3–11 ml of erythrocyte suspension until a value of approximately 0.60 was achieved. Next, a decrease in haematocrit (usually to a value of 0.2–0.1) was obtained by withdrawing blood and substituting with plasma or saline. Simultaneous measurements of haematocrit, CBF and arterial blood gases were performed. Hyperglycaemia was induced in control rats by intraperitoneal injection of 5–6 ml kg^{-1} body weight of 50% glucose hypertonic solution. Blood glucose measurements were performed at the beginning of the experiment and regularly after the glucose injection. Blood glucose values rose steadily in the following approximately 20 min and CBF measurements were performed within this period. A linear regression analysis was performed on the corrected CBF values and the inverse of the corresponding haematocrit in each diabetic and control rat, since a hyperbolic relationship between the two variables was expected[3] (see also discussion). Slopes and intercepts of the regression lines, as well as baseline CBF and blood glucose values, haematocrit, pCO_2 levels, and body weights from the two groups were compared, using the Mann–Whitney rank test. The statistical significance limit was $p < 0.05$. In the acute hyperglycaemic group, it was not possible to determine the relationships between blood glucose levels and CBF in the individual rats. Consequently, the data were pooled and the relative CBF changes were related to blood glucose values, by means of linear regression analysis.

Results

Body weight for all rats at the time of the experiment ranged between 260 and 450 g (334 ± 42, mean ± S.D.). After three weeks duration of diabetes, all STZ-treated rats had reduced body weights, with an average loss of 10.6%, whereas the normoglycaemic controls had a moderate weight gain. The baseline values of physiological parameters, measured on the day of the experiment, are shown in Table 1. Blood glucose levels in the diabetic group were significantly elevated throughout the study ($p < 0.01$).

In both groups a significant correlation between CBF and the inverse of haematocrit was found; in one rat only four concomitant measurements could be obtained and the correlation in that single rat could therefore not attain statistical significance ($0.1 < p < 0.05$). An example of the relationship between CBF and haematocrit is shown in Figure 1. The mean intercept and slope values of the regression lines did not differ significantly between the diabetic and the normoglycaemic groups, the relation being CBF = −46.5 ml 100 g min^{-2} + 65.4 haematocrit ml 100 g min^{-2} in the diabetic group and CBF = −62.5 ml 100 g min^{-2} + 72.8 haematocrit ml 100 g min^{-2} in the control group. In the acutely hyperglycaemic rats a significant and inverse relationship was found between CBF and plasma glucose ($r = 0.24$; $p < 0.001$): CBF = 70 ml 100 g min^{-2}, −0.8 blood glucose (in mM) (Fig. 2). Haematocrit remained constant throughout the whole experimental procedure: mean haematocrit (± S.D.) was 0.47 ± 0.03 before the glucose injection, and 0.46 ± 0.03 at the end of the hyperglycaemic period.

Discussion

In the present study the normal relationship between haematocrit and CBF is assessed in the rat. It is found that the CBF response to changes in haematocrit is preserved in STZ diabetes and, finally, in acute hyperglycaemia an inverse relationship between CBF and blood glucose is demonstrated. The dependency of CBF on haematocrit is caused by changes in arterial oxygen content; it has been demonstrated that the CBF alterations occurring during haemodilution parallel, and are fully explained by, the reduction in oxygen binding capacity. The increase in CBF is regulated so that the rate of oxygen delivery to the brain, defined as the product of arterial oxygen content and CBF, is kept constant.[3,13,14] This implies the existence of local mechanisms sensitive to tissue pO_2 levels.[15] This is further supported by our finding of a hyperbolic relationship between CBF and haematocrit.

Our results agree with and extend an earlier study where CBF was measured during moderate haemodilution using the ^{14}C-iodoantipyrine autoradiographic method in both normal and in 3–5 weeks STZ diabetic rats.[16] In our study, haematocrit was varied in both directions and to more extreme values (typically 0.60 to 0.10), and our experimental set-up allows for repeated measurements in the same animal, thereby reducing the

Table 1. Baseline values of physiological parameters on the day of the experiment

Group	(*n*)	Body weight (g)	Blood glucose (mM)	pCO_2 (mmHg)	Hct	CBF (ml 100 g min^{-2})
Diabetes	8	295 ± 22*	31.3 ± 4.7*	38.2 ± 2.6	0.45 ± 0.02	71.8 ± 22.4
Control	7	374 ± 39	7.8 ± 1.9	39.5 ± 2.9	0.47 ± 0.02	75.6 ± 13.6

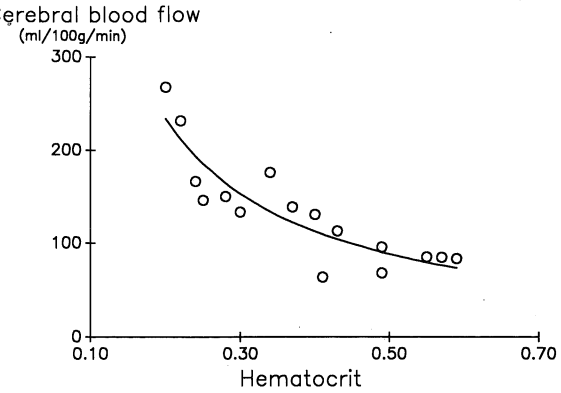

FIG. 1. Example of the inverse relationship between CBF and haematocrit in one STZ diabetic rat. The relationship is here CBF = − 9.1 ml 100 g min^{-2} + 48.6 Hct ml 100 g min^{-2}.

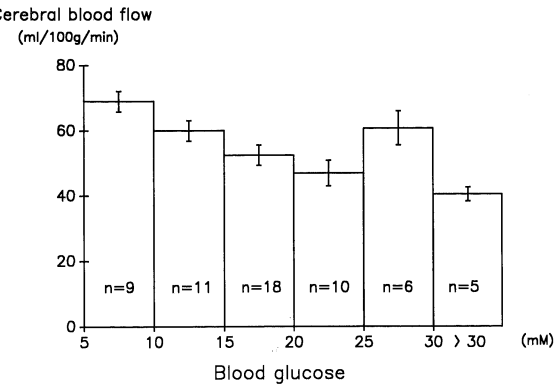

FIG. 2. CBF as a function of blood glucose in the acutely hyperglycaemic group. Each histogram represents blood glucose values in 5 mM intervals. CBF is given as the average ± s.e.m.

nherent variability and securing that the relationship could be established in individual rats. Our results show that the CBF-haematocrit relationship is preserved in STZ-diabetes, and that pO_2 reactivity of the cerebral arterioles therefore remains intact. In the STZ-diabetic group, the baseline CBF value was not significantly different from that of controls. In diabetic animals, both unchanged[8,17] and decreased[7,18] CBF has been reported, whereas in acute hyperglycaemia a reduction in CBF is usually found.[6,7,16,18,19] All previous studies have been using the autoradiographic method, whereas the intracarotid Xenon technique was used in the present study. No significant difference in baseline haematocrit values between diabetic and control rats was found, which is in accordance with other reports.[16,18,20,21]

In acute hyperglycaemia, Duckrow *et al*[6] suggested that blood glucose levels greater than 23 mM are necessary to produce a significant decrease in CBF; this statement is not confirmed by the present data, where a constant decline in CBF was observed with increasing blood glucose. In the same paper, a significant decrease in CBF was found only in 9 out of 24 cerebral regions, and 6 of these 9 were located in the hindbrain. The dose-response relation between regional CBF and blood glucose has been found to be linear; about − 7% for each 10 mM of glucose elevation, this correlation regions.[6,7] We measured global CBF, which is mostly determined by cortical flow values. The decreasing effect of hyperglycaemia on CBF may be relevant also in cerebral ischaemia: the hypothesis that the increased morbidity following ischaemic brain injury associated with hyperglycaemia[22] may be caused or augmented by decreased CBF has been forwarded.[6]

The mechanisms by which acute or chronic hyperglycaemia affect CBF might well be different. Increased osmolality, increased blood viscosity, and reduced glucose metabolism have been advocated as causative factors. In acute hyperglycaemia, cerebral glucose metabolism has been reported increased[17] or normal,[23] whereas chronic hyperglycaemia (> 3 weeks) leads to a decrease in glucose utilization.[17,23] None of the above mentioned factors, taken alone however, are able to explain the decrease in CBF.[6,7] Adaptive or compensatory changes may also occur in chronic hyperglycaemia, possibly accounting for unchanged baseline values in our diabetic group. In conclusion, our findings stress the importance of controlling haematocrit and blood glucose levels in the investigation of CBF in both normal and diabetic rats.

Conclusion

The present study demonstrates that CBF is inversely correlated with acutely increasing blood glucose levels, and that the CBF response to changes in haematocrit is preserved in experimental diabetes. Hyperglycaemia thus independently impairs CBF, but the mechanisms by which this effect is mediated remain to be clarified.

References

1. Dandona P, James IM, Newbury PA *et al. Br Med J* **2**, 325–326 (1978).
2. Griffith DNW, Saimbi S, Lewis C *et al. Diabetic Med* **4**, 217–220 (1987).
3. Henriksen L, Paulson OB and Smith RJ. *Ann Neurol* **9**, 454–457 (1981).
4. Hudak ML, Koehler RC, Rosenberg AR *et al. Am J Physiol* **251**, H63-H70 (1986).
5. Harrison MJG. *Cerebrovasc Metab Brain Res* **1**, 55–67 (1989).
6. Duckrow RB, Beard DC and Brennan RW. *Ann Neurol* **17**, 267–272 (1985).
7. Duckrow RB, Beard DC and Brennan RW. *Stroke* **18**, 52–58 (1987).
8. Knudsen GM, Göbel U, Paulson OB *et al. J Cereb Blood Flow Metab* **11**, 361–365 (1991).
9. Maruyama M, Shimojo K, Ichikawa T *et al. Stroke* **16**, 675–679 (1985).
10. Hemmingsen R, Hertz MM and Barry DI. *Acta Physiol Scand* **105**, 274–281 (1979).
11. Olesen J, Paulson OB and Lassen NA. *Stroke* **2**, 519–540 (1971).
12. Chen RYZ, Fan F-C, Kim S *et al. J Appl Physiol* **49**, 178–183 (1980).
13. Häggendal E, Nilsson NJ and Norbäck B. *Acta Chir Scand* **364** (suppl), 3–12 (1966).
14. Paulson OB, Parving H-H, Olesen J *et al. J Appl Physiol* **35**, 111–116 (1973).
15. Brown MM, Wade JPH and Marshall J. *Brain* **108**, 81–93 (1985).
16. Duckrow RB. *Stroke* **21**, 1072–1076 (1990).
17. Jakobsen J, Nedergaard M, Aarslew-Jensen M *et al. Diabetes* **39**, 437–440 (1990).
18. Harik SI and LaManna JC. *J Neurochem* **51**, 1924–1929 (1988).
19. LaManna JC and Harik SI. *J Cereb Blood Flow Metab* **3** (Suppl. 1), S409–S410 (1983).
20. Rubin MJ and Bohlen HG. *Am J Physiol* **249**, H540–H546 (1985).
21. Kikano GE, LaManna JC and Harik SI. *Stroke* **20**, 1027–1031 (1989).
22. Pulsinelli WA, Waldman S, Rawlinson D *et al. Neurology* **32**, 1239–1246 (1982).
23. Duckrow RB and Bryan RM. *J Neurochem* **49**, 989–993 (1987).

ACKNOWLEDGEMENTS: The authors wish to thank Karin Stahr for her excellent technical assistance. This study was supported by the Danish Diabetes Foundation and by the Danish Medical Association (Højmosegårdlegatet).

Received 20 August 1992;