Brain metabolism after recurrent insulin induced hypoglycaemic episodes: a PET study

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Abstract

Neuropsychological testing was carried out and the rate of oxygen metabolism in the brain was measured by PET in 15 highly selected patients with type 1 diabetes. The aim was to investigate the impact on the brain of hypoglycaemic comas resulting from insulin treatment. No significant difference was found between nine patients with a history of more than 10 hypoglycaemic comas and six others who denied any history of such events. These data suggest that intensified insulin treatment, although increasing the frequency of hypoglycaemic coma, may not always be harmful for the brain. This may be explained by the limited duration of hypoglycaemic coma induced by conventional insulin treatment.

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Accurate glycaemia control by insulin treatment may prevent or delay most of the long term complications of diabetes mellitus.¹ As a consequence, hypoglycaemia is now the most frequent morbid event in insulin dependent diabetic patients as the optimal glycaemic control increases the risk of hypoglycaemia two to threefold.² Some 10-25% of patients with type 1 diabetes have at least one episode of severe hypoglycaemia, often with seizure or coma, in a given year.³ This raises the problem of the long term impact of insulin induced hypoglycaemic episodes on the brain.⁴ Several neuropsychological studies have previously considered this point. Some results, but not all, are consistent with a cumulative cognitive impairment after recurrent hypoglycaemic episodes.² To further investigate this issue, we studied the cerebral metabolic rate of oxygen (CMRO₂), brain MRI, and cognitive function in highly selected patients with insulin dependent diabetes. We compared patients with a history of recurrent hypoglycaemic comas with patients who denied any history of such events.

Materials and methods

PATIENTS

Fifteen patients with insulin dependent diabetes gave their informed consent to participate in the study. They were selected according to the criteria: (a) lack of macro-

angiopathic complications of diabetes based on normal arterial blood pressure, no history of cerebrovascular disease, normal CT, and normal vascular examination by cervical and transcranial doppler; (b) lack of major microangiopathy based on normal visual acuity with no proliferative retinopathy and albuminuria less than 300 mg/24 h; (c) lack of postural hypotension, peripheral sensory or motor deficit; (d) either a history of more than 10 hypoglycaemic comas or no history of severe hypoglycaemia (requiring assistance from others).

Nine patients (group HC) reported more than 10 hypoglycaemic comas (episodes of coma rapidly relieved by treatment with glucagon or intravenous glucose). Detailed history concerning these events was recorded on the basis of interviews of patients and witnesses (spouses or equivalent), and on medical records. In all of these patients, hypoglycaemia unawareness (failure to recognize autonomic warning symptoms or lack of symptoms before development of neuroglycopenia⁵) has been previously recorded in hospital. This condition is known to increase the risk of hypoglycaemia 25-fold and to cause severe hypoglycaemic insults.⁵⁻⁶ The exact number of episodes experienced in a lifetime was known in six subjects (range 10-20) but was not precisely determined in three subjects because of their high number (range ≈ 50-100). In each patient, an apparently complete clinical recovery from hypoglycaemic insults was always seen within less than one hour of dextrose or glucagon injection and none had previously required admission to an intensive care unit.

Six patients without any history of hypoglycaemic comas (group NHC) were similarly investigated. Hypoglycaemia unawareness had never been documented in these patients.

Groups HC and NHC did not differ in mean age (mean (SE)) (range): 47.6 (4.4) (30 to 71) v 42.83 (7.72) (20-63) years, educational level, duration of diabetes (19.56 (4.06) (3-38) v 11.33 (2.85) (2-21) years), daily dose of insulin (34.8 (3.38) (17.5-45) v 44.1 (8.6) (12-75) U), or glycosylated haemoglobin concentration (7.24 (0.45) (4.8-9.7) v 7.05 (0.33) (6.3-7.9)%: normal range 4-5.6%).

For all patients, the neuropsychological testing was performed under their usual regimen of insulin treatment during the week preceding the PET study and when they were admitted to hospital. All of them underwent

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frequent blood glucose estimations in the 24 hours before testing either neuropsychologically or with PET. No episodes of hypoglycaemia were noted 24 hours before cognitive or PET examination.

NEUROPSYCHOLOGICAL TESTS

Blood glucose concentrations were measured with a glucometer immediately before and after testing. The values ranged from 6 to 11 mmol/l.

The neuropsychological examination included tests aimed to assess global efficiency, attention, memory, and verbal fluency.⁷ These tests are highly sensitive and can be used in groups of small size.⁷

Global efficiency was screened by the trail making test and the letter cancellation test. The trail making test assesses visual scanning and motor planning on motor speed and attention. This test is given in two parts, A and B. In part A, the patient must connect a series of randomly arrayed numbers in numerical sequence (1–2–3 . . .). In part B, numbers and letters must be connected in alternating sequence (1-A, 2-B, 3-C . . .). We scored this test by the time needed to complete the task.⁷ The letter cancellation test requires visual selectivity at fast speed on a repetitive motor task. Subjects are presented with letters and must cancel one given letter as fast as possible. The score is dependent on the time taken for the task and the percentage of errors.

The Stroop word task is a classical attentional task. Although the type of brain circuit used in attention is still debatable, recent PET studies have suggested that this task mainly involves the cingulate gyrus and the frontal cortex.^{8–9} The test requires the subject to read or name the colours from a list under three conditions: (a) read colour names printed in black ink; (b) name the colours presented in coloured dots; (c) name the colour of ink in which incongruous colour names are printed (for example, “RED” printed in green ink, the subject names the ink colour “green”). The time elapsed is evaluated for each task. This test provides a measure of interference of word reading on colour naming.

Memory tests included the digit span and auditory verbal learning test. The digit span of the Wechsler adult intelligence scale (WAIS) verbal scale explores the immediate repetition of growing series of digits in direct order (A) and in reverse order (B). This is a test of attention and short term memory. The auditory-verbal learning test⁷ consists of five presentations of a list of 15 words with immediate recall and a single delayed recall 30 minutes later. Words are read at the rate of one per second. We scored the mean number of words recalled at the five first recall trials and the number of words recalled at the delayed trial.

Verbal fluency was assessed with the lexical fluency test. This test consists of the oral production of the maximum number of words beginning with a designated letter during one minute. The total number of words given was used as the score. Studies with PET suggest

that the left frontal lobe and cingulate gyrus are involved during this task.¹⁰

MAGNETIC RESONANCE IMAGING

Cerebral MRI was performed on the same day as the PET study on a superconducting 0.5 tesla General Electric imager. Ten to 12 contiguous sections (5 mm thickness) were acquired parallel to the orbitomeatal line. Head positioning was verified with laser beams. Cutaneous landmarks were drawn to allow an exact repositioning of the head during the PET study. T1 (TR = 480 ms; TE = 12 ms) and T2 (TR = 2300–2900 ms; TE = 100 ms) weighted sequences were obtained for all subjects. Images were independently analysed by a neurologist blinded to the age and state of the patients. Areas of increased signal intensity were scored on T2 weighted images using the scheme reported by Bowen *et al.*¹¹ The degree of cortical atrophy was ranked based on the sulci aspect on the T1 weighted MR images obtained at the orbitomeatal line + 40 mm by three of us (HC, ML, YS) who were blinded to the subjects' condition. The final rank was based on the sum of the three scores.¹²

POSITRON EMISSION TOMOGRAPHY

The subjects continued their usual regimen before the PET study. The study started at 10 00 am, after the morning insulin injection and breakfast between 7 30 and 8 30 am. Cerebral blood flow (CBF), oxygen consumption rate (CMRO₂), cerebral blood volume (CBV), and oxygen extraction fraction (OEF) were measured with the ¹⁵O inhalation method using successive inhalation of C¹⁵O₂, ¹⁵O₂, and C¹⁵O.^{13–14} The positron camera used was a 4 ring LETI-TTVO1 time of flight camera with both slice thickness and lateral resolution of 12 mm. Seven slices were obtained (four direct and three cross sections with an interslice space of 3 mm).¹⁵ Correction for attenuation was carried out with ⁶⁸Ge-⁶⁸Ga transmission scans before gas inhalation. The studies were performed with the patients at rest, their eyes closed, and their heads positioned as in MRI, using the cutaneous landmarks and a crossed laser beam system. The lowest slice was located 10 mm above and parallel to the orbitomeatal line. During each of the three successive scans, blood samples were withdrawn from a radial artery catheter to measure the ¹⁵O radioactivity (two samples) in whole blood and plasma and the PaO₂, PaCO₂, pH, haematocrit, haemoglobin, and glucose values. The CMRO₂ was calculated with correction for the intravascular labelled oxyhaemoglobin using the C¹⁵O steady state image (CBV).¹⁶

Four slices were used for data analysis. They corresponded respectively to the “cerebellar cut” (orbitomeatal line + 10 mm), “basal ganglia cut” (orbitomeatal line + 40 mm), “low centrum semiovale cut” (orbitomeatal line + 55 mm) and “high centrum semiovale cut” (orbitomeatal line + 70 mm). A standardised and already validated method for the regional cortical data analysis was

Regional CMRO₂ indices in HC group (solid circles) and NHC group (open circles) in large lobar (A) and MRI based smaller (B) cortical regions and in subcortical regions (C).

used.¹⁷ Circular regions of interest of 3 cm² were positioned on the CBF images along the cortical rim of one cerebral hemisphere tangentially to each other and to an external isocontour of 30% of maximal activity. These regions of interest were then mirror copied on the opposite hemisphere. They were pooled in six large lobar regions corresponding to the frontal, temporal, parietal, and occipital cortex. Lobar limits were based on the atlas of Matsui and Hirano¹⁸ and individual MRI. In addition, putaminal, thalamic, mediotempo-

ral, and anterior cingulate regions of interest were drawn on MRI and superimposed on the corresponding CBF images after verifying that the contour of the brain obtained on MRI corresponded to the 30% isocontour of CBF images at the same levels. All regions of interest were subsequently translated on the corresponding CMRO₂, OEF, and CBV images.

STATISTICS

The Mann-Whitney U test was used to compare the ranking for cortical atrophy and the scores obtained on MRI signal abnormalities between the groups. The same test was used to compare each score of the neuropsychological assessment between HC and NHC groups.

A two tailed unpaired Student's *t* test with appropriate Bonferroni correction was used for PET data analysis. Mean cortical absolute values of CBF, CMRO₂, OEF, CBV, and CBF/CBV and regional "metabolic indices" obtained by dividing each regional CMRO₂ values by the mean cortical CMRO₂ were compared between the groups.

Results

MAGNETIC RESONANCE IMAGING

The ranking obtained for cortical atrophy did not show any difference between the two groups. Hence, cortical atrophy was visually estimated as minimal or absent in all patients, except one who had moderate sulcal enlargement.

The scores obtained for MRI signal abnormalities did not differ significantly between the two groups. Also, the scores were within the normal range according to the criteria of Bowen *et al.*¹¹ No signal abnormality was detected on T1 images. No signal abnormality was seen on T2 images in the NHC group. Only a few punctate hypersignals, always less than 2 mm in size, were found and occurred in four of nine HC group patients. These hypersignals were located in the periventricular area in one case (two lesions in one 71 year old patient) and outside the periventricular domain in four cases (10 lesions in the 71 year old; three, three and one lesions respectively in the other cases). Similar signal abnormalities are often seen in normal elderly subjects.

NEUROPSYCHOLOGICAL TESTS

The neuropsychological testing did not show any statistically significant difference between HC and NHC groups (table 1).

POSITRON EMISSION TOMOGRAPHY

The PET results did not show any significant difference between the HC and NHC groups. The mean cortical values of CBF, CMRO₂, OEF, CBV, and CBF/CBV did not differ between the groups (table 2); these values did not differ significantly from the corresponding values obtained in six healthy subjects of similar mean age who had neither MRI nor glucose measurement and who were studied during the same year in our centre. No difference was found between the HC and NHC

Table 1 Neuropsychological results

	HC group median (range)	NHC group median (range)
Trial making test (s):		
Part A	116.1 (80.8–297.5)	109.6 (42.7–142.5)
Part B	115.1 (85.1–228.4)	122.1 (60.0–185.1)
Letter cancellation:		
Time for the task (s)	62.1 (33.5–92.7)	56.9 (46.1–83.2)
Errors (%)	6.5 (0–19.5)	6.5 (0–10.9)
Stroop word task (s):		
Part 1 (colour naming)	26.4 (18.7–36.9)	23.1 (19.8–29.6)
Part 2 (no interference)	41.1 (31.5–53.3)	44.4 (35.6–47.5)
Part 3 (interference)	79.0 (60.0–119.9)	81.5 (63.8–119.2)
Digit span:		
Direct order	6 (5–7)	5.5 (5–6)
Reverse order	4 (3–6)	4 (3–5)
Auditory verbal learning test:		
Mean of 5 recall trials	10 (8–12)	10 (8–12)
6th delayed recall trial	11 (7–15)	13 (6–15)
Lexical fluency	8 (3–14)	12 (9–18)

There was no significant difference between HC group and NHC group for each score obtained.

Table 2 Mean absolute values of CBF, CMRO₂, OEF, CBV, and CBF/CBV calculated for the whole cortex in HC and NHC groups

	Group HC	Group NHC
CBF (ml/100ml/min)	31.1 (24.33–38.21)	31.2 (22.65–39.53)
CMRO ₂ (ml/100ml/min)	2.41 (1.92–2.62)	2.27 (2.05–2.77)
OEF (%)	0.46 (0.32–0.52)	0.42 (0.35–0.57)
CBV (ml/100ml)	3.38 (2.58–5.17)	3.49 (3.03–3.66)
CBF/CBV (1/min)	9.93 (6.13–12.34)	9.23 (7.35–12.32)

Values in parentheses are 95% confidence intervals. CBF = cerebral blood flow; CMRO₂ = oxygen consumption rate; OEF = oxygen extraction fraction; CBV = cerebral blood volume.

groups for the different regional CMRO₂ indices (figure). During the PET study, the two groups did not differ in glycaemia (9.01 (95% confidence interval 5.3–15.2) v 11.03 (7.68–14.32) mmol/l), packed cell volume (42 (39–46)% v 42 (37–46)%, pH (7.39 (7.38–7.40) v 7.39 (7.38–7.40) or pCO₂ (39.24 (35.95–42.53) v 39.76 (36.85–42.68) mm Hg).

Discussion

The aim of this study was to determine whether hypoglycaemic comas resulting from insulin treatment in diabetes can harm the brain. We did not find any neuropsychological, MRI, or PET evidence to support such a detrimental role for these comas. By contrast, several authors previously reported deleterious consequences of repeated severe hypoglycaemic insults on cognitive function in patients with insulin dependent diabetes. Wredling *et al* found that type 1 diabetic patients with recurrent severe hypoglycaemia scored lower in tests of motor ability, short term and associative memory, and some visuospatial tasks.¹⁹ They suggested that such a pattern might indicate a frontal dysfunction. These findings were in line with those of Bale *et al* and Sachon *et al* who showed that patients with recurrent severe hypoglycaemia performed poorly in tests of auditory learning and short term memory.^{20–21} More recently, Langan *et al* reported significant correlations between the frequency of recurrent severe hypoglycaemia and measurements of the performance IQ of the Wechsler adult intelligence scale and of inspection time and reaction time.²² Our negative results may be related to the small size of our sample (β type statistical error). We consider this unlikely, however, for the following reasons. The median and mean values of the neuropsychological scores were similar in both groups. In a recent three year prospective study, Reichard *et al* did not find any effect of intensified insulin treatment on cognitive function despite more frequent recurrent severe hypoglycaemia.²³ Because the measurement of brain metabolism has been shown to have high sensitivity for detection of cerebral abnormalities in small series of patients with ischaemia,²⁴ dementia,^{25–26} or epilepsy,²⁷ the lack of metabolic abnormality in our study further supports the idea that recurrent hypoglycaemic comas can leave the brain undamaged in patients with insulin dependent diabetes. It may well be that this is true, however, only in the absence of angiopathic com-

plications as our study was designed to exclude these. Indeed, Ryan *et al* recently reported that only in patients with insulin dependent diabetes with neuropathy does recurrent hypoglycaemia worsen intellectual performance.²⁸ Multiple brain injuries have been reported in neuropathological studies after hypoglycaemic comas. Kalimo *et al* found an extensive necrotising injury with gliosis in the cerebral cortex, amygdala, hippocampus, putamen, globus pallidus, and thalamus in a patient who died after two months in a post-hypoglycaemic coma.²⁹ Moersh *et al* reported two patients with insulinomas who died after a prolonged hypoglycaemia.³⁰ Both of these patients had neuronal degeneration in the cortex and basal nuclei. Diffuse and widespread brain damage has also been reported after fatal hypoglycaemia in babies.³¹ In another study, Iwai *et al* reported a periventricular hypodensity and pronounced contrast enhancement of the cortex with CT in one patient in a persistent vegetative state after insulin suicide.³² In all these cases, recovery after the resolution of hypoglycaemia usually took several hours or days and was most often incomplete, leaving residual brain dysfunction as severe motor deficits, dementia, or prolonged coma, followed by death. Such hypoglycaemic comas were profound, and of a very long duration, because they resulted from insulinomas, insulin suicide, or overdose of long acting hypoglycaemic agents.^{33–34} Hence, the lack of persistent cognitive, MRI, and metabolic alterations in our patients may be the result of therapeutic doses of insulin with time limited hypoglycaemic effects, and, perhaps, of maintained counter-regulatory hormonal secretions.³⁵ The striking importance of the duration of hypoglycaemia is supported by experimental studies, which show a clear relation between the duration of the insulin induced hypoglycaemic comas and the severity of the resultant brain damage. Auer *et al* showed that the irreversible brain damage after hypoglycaemia in rats required at least one hour of flat EEG.³⁶ Light and electron microscopic examination showed that after this duration, brain damage affected only the superficial laminae of the neocortex (chiefly layer 2), the hippocampal gyri, and the striate nucleus. The neuronal changes in the neocortical layers 4 and 6 were reversible.^{37–39} Furthermore, when this delay had passed, the brain damage correlated with the EEG isoelectric time.⁴⁰ In monkeys, 57 minutes of absent evoked potentials (corresponding to 10–20 minutes of flat EEG) were not enough to produce brain damage.⁴¹ Our data support the view that a “brain damage free period” also exists during hypoglycaemic comas in humans. Whereas we found that this period was less than one hour in most of our cases, a more exact estimate was impossible as the duration of the comas could not always be precisely ascertained. The prolonged brain resistance to hypoglycaemia contrasts with the immediate brain damage caused by hypoxia^{17–42} or ischaemia.⁴³

This suggests that endogenous non-glucose fuels may be used by the brain to maintain the cellular energy state for a limited period. In support of this, the brain oxygen consumption was found to be maintained close to normal values during 15 minutes of flat EEG⁴⁴ whereas the glucose to oxygen utilisation⁴⁴ was greatly reduced in hypoglycaemic rats. Furthermore, the ATP level only dropped to 30% during this period, contrasting with the ATP decrease of 95% during hypoxia.⁴⁵⁻⁴⁷ In humans, Della Porta *et al* also found that the brain oxygen consumption underwent a slight but insignificant diminution during the sakel insulin treatment, whereas the cerebral glucose utilisation decreased progressively by more than 80%. This result was obtained 90 minutes after insulin injection, during a state of somnolence, and four to five hours later, during a profound coma.⁴⁸ Thus our results suggest that this adaptative metabolic state may effectively protect the human brain during short insulin induced hypoglycaemic events.

In summary, the combined MRI, PET, and neuropsychological data obtained in a small but highly selected group of patients indicate that the brain is resistant to the hypoglycaemia that may occur during insulin treatment. These data may be helpful in the design of large clinical studies to investigate the risk:benefit ratio of intensified insulin treatment.

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