INSUFFICIENT ADAPTATION OF HYPOGLYCAEMIC THRESHOLD FOR COGNITIVE IMPAIRMENT IN TIGHTLY CONTROLLED TYPE 1 DIABETES

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SUMMARY - It is well known that hypoglycaemic thresholds for hormones and symptoms occur at lower plasma glucose levels in patients with strict glycaemic control. However, whether the threshold for cognitive impairment also shifts is still an unresolved question. We studied 19 type 1 diabetic patients, including 8 with hypoglycaemia unawareness, aged 37.0 ± 7.4 y.r., with diabetes duration 15.2 ± 10.7 yr, and HbA1c 7.6 ± 1.1%. Hypoglycaemic thresholds for hormones, symptoms, awareness and cognitive function using the 4-choice reaction time test (4RT), were measured every 30 min during a 150 min stepped 4.4 to 2.2 mM hypoglycaemic hyperinsulinemic clamp. We found that 4RT- accuracy deteriorated earlier than 4RT-time (3.2 and 2.7 mM, respectively, p < 0.01), and that both correlated poorly with HbA1C before and after adjustment for age and diabetes duration (r = 0.11, and 0.18, respectively). On the opposite, site, adrenaline, autonomic and neuroglycopenic symptoms, and awareness significantly correlated with HbA1C values (r = 0.56, 0.70, 0.61, and 0.63, after adjustment, respectively). Furthermore, after allocating the patients into two subgroups according to HbA1C values (< 8% n = 12, and ≥ 8% n = 7), we found that, as opposed to other thresholds, accuracy and 4RT-time were minimally and not significantly influenced by glycaemic control, therefore exhibiting the smaller glucose thresholds shifts. In conclusion: 1) the hypoglycaemic thresholds for cognitive dysfunction shift with strict glycaemic control, but not significantly and less than other thresholds, 2) as opposed to other reports, accuracy deteriorated earlier than speed during the 4RT test, and 3) these “mal-adapted” reactions may contribute to the higher risk for severe hypoglycaemia in subjects with tight glycaemic control.

Key-words: hypoglycaemia, cognitive function, 4-choice reaction time, counterregulation, diabetes.

RÉSUMÉ - Adaptation insuffisante du seuil hypoglycémique de détérioration cognitive chez le diabétique de type 1 strictement équilibré. Les seuils hypoglycémiques de déclenchement des réactions et des symptômes surviennent pour des taux glycémiques plus bas chez les patients strictement équilibrés. Un même type de dérive pour le seuil de détérioration cognitive reste encore une question controversée. Nous avons étudié 19 patients diabétiques de type 1, dont 8 avec hypoglycémie non ressentie, âgés de 37.0 ± 7.4 ans, présentant un diabète depuis 15.2 ± 10.7 ans, et une hémoglobine glycosylée à 7.6 ± 1.1%. Les seuils glycémiques pour les hormones, les symptômes, la perception, et les fonctions cognitives testées par le test 4RT (temps de réaction à une stimulation lumineuse avec 4 choix) ont été mesurés toutes les 30 minutes pendant les 150 minutes qu’a duré le clamp hypoglycémique hyperinsulinémique (4.4 à 2.2 mmol). Résultats : l'exactitude au 4RT s’est détériorée plus tôt que la vitesse de réaction au 4-RT (3.2 et 2.7 mmol respectivement, p < 0.01). Ces deux paramètres du test montrent une mauvaise corrélation avec l’hémoglobine glycosylée avant et après ajustement pour l’âge et la durée du diabète (r = 0.11 et 0.18 respectivement). A l’opposé, l’adrénaline, les symptômes dysautonomiques et neuroglucopéniques et la perception de l’hypoglycémie étaient corrélés significativement avec l’hémoglobine glycosylée (r = 0.56, 0.70, 0.61 et 0.63 après ajustement respectivement). De plus, après avoir réparti les patients en deux sous-groupes selon leur équilibre glycémique (hémoglobine glycosylée < 8 %, n = 12, et hémoglobine glycosylée ≥ 8 %, n = 7), il s’est avéré que contrairement aux autres seuils, l’exactitude et la vitesse de réaction au 4-RT sont non significativement influencées par le degré de contrôle glycémique, avec la dérive vers le bas la plus faible (r = -0.2 et - 0.5 mmol pour l’exactitude et la vitesse respectivement, vs 0.6 à 0.8 pour les autres seuils). Conclusion: 1) le seuil hypoglycémique de détérioration cognitive dérive vers le bas avec le strict contrôle glycémique, mais moins et non significativement, à l’opposé des autres seuils, 2) contrairement aux données de la littérature, l’exactitude se détériore plus tôt que la vitesse au cours du test 4-RT, et 3) ces réactions mal adaptées peuvent contribuer au risque accru d’hypoglycémie sévère chez les sujets en strict contrôle glycémique.

Mots-clés : hypoglycémie, fonction cognitive, test de réaction à la stimulation lumineuse avec 4 choix (4-RT), contre-régulation, diabète.
The evidence that strict glycaemic control delays the development and progression of diabetic microvascular complications has prompted the wider use of intensified insulin therapy [1-3]. This therapeutic approach increased the frequency of severe hypoglycaemic episodes by three-fold in the largest studies [4, 5]. To date, there is a good agreement that antecedent hypoglycaemia, possibly by the way of hypothalamic desensitization, not only reduces intensity of counterregulation but also shifts the glycaemic thresholds for activation of autonomic counterregulation and initiation of awareness of hypoglycaemia to lower plasma glucose concentrations. These two mechanisms contribute to the frequency and severity of hypoglycaemia and induce a vicious cycle [6-12]. Some subjects, including patients with long-standing diabetes, have a deeper counterregulatory dysfunction, leading to an hypoglycaemia unawareness syndrome, a state in which affected patients no longer have the warning symptoms of developing hypoglycaemia that previously prompted them to take action, e.g. to eat, to prevent progression to severe hypoglycaemia [13-16].

In all above circumstances, it is not clear whether such a shift also occurs with the glycaemic threshold for cognitive dysfunction [17-23]. In various hypoglycaemic conditions, especially in type 1 diabetic patients with good glycaemic control or antecedent hypoglycaemia, some studies using a battery of neuropsychological tests [24, 25] or the P300 potential [26] have described a downshift of the cognitive impairment threshold (“adaptation”), whereas others using only one test (4-choice reaction time, 4RT(, or EEG [27, 28], or a battery of tests [29, 30] have found “fixity of cognitive impairment threshold”.

The controversial issue of fixity or adaptation of cognitive impairment thresholds is clinically relevant because the downward shifting of the autonomic and awareness thresholds might have more deleterious consequences if cognitive dysfunction thresholds do not shift parallel to others, i.e. cognitive impairment precedes the appearance of symptoms, thus rendering subjects unable to take appropriate measures to promptly correct hypoglycaemia [18-23].

Therefore this study was undertaken to determine the influence of glycaemic control on plasma glucose threshold for initiating cognitive dysfunction. For this purpose, cognitive functions were assessed using 4RT in type 1 diabetic patients with variable degrees of glycaemic control during a stepped hypoglycaemic hyperinsulinemic clamp.

**SUBJECTS, MATERIALS AND METHODS**

**Subjects** – 19 type 1 diabetes patients, aged 25-50 years, were studied, including 8 with hypoglycaemia unawareness according to Cox Questionnaire [16]. This questionnaire includes 7 questions which explore the frequency and severity of misperceived hypoglycaemias over the past year. Twelve of these patients had good glycaemic control (HbA1c < 8%, normal values ≤ 6.0%) whereas others were fairly controlled (HbA1c ≥ 8%). Eligibility for study included diabetes duration of at least 2 years, no clinical autonomic neuropathy (based on symptoms, e.g., orthostatic hypotension and diarrhea), no system medications other than insulin and no acute illness. The clinical characteristics of subjects are shown in Tables IA and B. Each gave written informed consent to participate in the study protocol, which was approved by the Human Subject Review Committee of Hôtel-Dieu Hospital, Paris.

**Hypoglycaemic clamp procedures** – Responses to hypoglycaemia were measured during a hypoglycaemic clamp performed after a 10- to 12-h overnight fast. Normoglycaemia was maintained overnight using frequent capillary glycaemic measurements. In case of hypoglycaemia, the test was postponed by ≥ 1 week. In the morning, usual insulin dose and breakfast were omitted, and the subjects were trained at least four times at performing 4RT in order to achieve stable recordings. Then, two intravenous catheters were inserted, one in an antecubital vein for infusion of glucose and insulin, and the other in a dorsal hand vein for blood sampling. This hand was placed in a heated
(60–65°C) box to arterialize venous blood. Rapid human insulin (Actrapid, Novo Nordisk) in a 0.9% sodium chloride was infused during a 60 min basal period to achieve euglycaemic levels, then set at a fixed rate of 1ml/Kg/min. Using a lap-top computer programme (J.J. Robert, Necker Hospital, Paris, unpublished), loaded regularly with the plasma glucose measurements, the infusion rate of 30% glucose was automatically adjusted every 3-5 min for plasma glucose to reach gradually 2.2 mM in 0.5-0.6 mM steps (4.4, 3.9, 3.4, 2.8 and 2.2 mM) after 120 min and to be maintained at this latter level for 30 min. On completion of the clamp, plasma glucose was restored to >8 mM, and the patients took a rest and ate their lunch before returning to their usual insulin treatment.

Measurements and calculations – All blood samples were centrifuged immediately at 4°C and the plasma was kept frozen at –20°C until analysed, except for adrenaline that was processed immediately. Blood glucose levels were measured by a glucose-oxidase method (Autoanalyser II, Beckman Instruments, Brea, CA, USA) at 3- to 5-min intervals. Additional blood was taken every 30 min, and every 15 min during the 2.2 mM hypoglycaemic plateau for free insulin, glucose and counterregulatory hormone measurements. Plasma free insulin levels were measured by radioimmunoassay (RIA- Pasteur, Paris, France) after immediate antibody extraction with polyethylene glycol [31]. Glucagon samples were collected into aprotinin-EDTA and measured by radioimmunoassay [32]. Cortisol was measured by radioimmunoassay on serum and growth hormone by immunoradiometric assay (K.G.M.M. Alberti, Newcastle, England, unpublished). Adrenaline samples were collected on reduced glutathion and EGTA and immediately measured by a radioenzymatic method [33].

Feeling of hypoglycaemia was recorded every 30 min, and every 15 min during the 2.2 mM hypoglycaemic plateau.

To evaluate hypoglycaemic symptoms, a 9-item questionnaire derived from Hepburn [34] were administered every 30 min, and every 15 min during the 2.2 mM hypoglycaemic plateau. The questionnaire included assessment of five autonomic and four neuroglycopenic symptoms, each one being scored according to intensity, from 0 (absent) to 7 (very severe). Autonomic symptoms scores were calculated from the scores of sweating, anxiety, trembling, feeling hot and heart pounding, and neuroglycopenic symptoms scores from the scores of inability to concentrate, blurred vision, tiredness and difficulty in speaking. Symptoms present at baseline were not counted in final scoring.

After the completion of each questionnaire, a 4RT test was administered using a Reaction Timer device (AMPD-PM-2217, Central Sheffield University Hospital, UK). Briefly, the device records the accuracy (number of errors), and time (milliseconds, ms) for the subject to clear a visual symbol appearing randomly in one of four quadrants of a computer screen, by pressing the corresponding key on a keypad [35, 36]. 500 measurements were made on each occasion and averaged.

The hormonal thresholds were defined as the glucose levels at which the hormone achieved a defined increment over the base-line, in at least two successive samples. The increments were: > 410 pmol/l for adrenaline, > 0.33 nmol/l for noradrenaline, > 192 nmol/l for cortisol, and > 18 mU/l for growth hormone. For glucagon, the threshold was defined as the glucose level at which glucagon exceeded the basal values by 2 SD, in at least 2 consecutive measurements. The above criteria have been used by other authors because they correspond to physiologically significant hormone responses to hypoglycaemia [37, 38].

A rise in symptom scores was considered if two points or more over the score at normoglycaemia, on at least two successive measurements. Threshold of awareness was set when the answer about feeling hypoglycaemic was affirmative, in at least two consecutive occasions.

Plasma glucose thresholds for cognitive dysfunction were determined in two ways: 1) in statistical terms, as a rise of at least 2 SD above mean basal values, on at least two successive recordings, and 2) as a change in performance test of known physiological significance. This change was defined as an increase over baseline of > 2% for speed, or of > 5% for accuracy, in at least two consecutive measurements, as recommended by Maran et al [27].

If a significant change did not occur during hypoglycaemia, the glucose nadir was entered as the threshold for the statistical procedures.

Statistical analysis – Except for population data, which are given as mean ± SD, all results are expressed as means ± SEM. The HbA1c values were correlated using partial correlation analysis (Mac JMP, version 3) with the counterregulatory thresholds obtained throughout the clamps, before and after adjustment for age and diabetes duration as potential confounding variables. Significance of comparisons between good and fair glycaemic control patients were assessed using unpaired Student’s t test.

### RESULTS

Glucose and insulin profiles. The hypoglycaemic clamp procedures reduced plasma glucose levels in a nearly similar fashion in each of the two populations (Fig. 1). The insulin infusion produced a sustained increase in plasma insulin levels (553 ± 35 pM) that were not statistically different between the groups. The results below were calculated on all patients, including 2 female patients in the well-controlled group, who did not significantly influence the findings (data not shown).

Counterregulatory hormones – Basal hormonal levels did not differ between the groups. On the whole population, hypoglycaemic threshold for adrenaline occurred at 2.5 ± 0.1 mM, and correlated significantly with HbA1c values, before (r = 0.54, p < 0.02) and after adjustment for age and diabetes duration (r = 0.56 p < 0.02). The good glycaemic control group had a delayed and reduced response to hypoglycaemia (threshold, T = 2.3 ± 0.2 mM, area under curve per 150 min, AUC = 770 ± 210 pM, peak of response = 2881 ± 544 pM). In contrast, the fair glycaemic control group began to secrete adrenaline at higher plasma glucose levels in higher quantities (T= 2.9 ± 0.3 mM, p= 0.04, AUC= 1780 ± 650 pM, NS, and peak of response = 5114 ± 1440 pM, NS). The statistically significant delayed response in the good glycaemic control group is not explained by the basal adrenaline levels. Secretions of other hormones, i.e.,
noradrenaline, glucagon, cortisol, and growth hormone occurred at lower blood glucose levels in the patients with strict glycaemic control than in those with fair glycaemic control, though differences were significant for growth hormone only (p < 0.05) (Table II).

**Hypoglycaemic symptoms and awareness** – On the whole population, hypoglycaemic thresholds for autonomic and neuroglycopenic symptoms occurred at 2.4 ± 0.2 and 2.8 ± 0.2 mM, respectively, while hypoglycaemia was perceived at 2.6 ± 0.2 mM. Thresholds for autonomic and neuroglycopenic symptoms, and awareness, showed strong correlation with diabetes control, before and after adjustment for age and diabetes duration (r = 0.70 p < 0.01, r = 0.61 p < 0.01 and r = 0.63 p < 0.01, respectively). The rise in autonomic symptoms began at lower blood glucose levels in tightly controlled patients than in those with fair glycaemic control (T = 2.1 ± 0.1 vs 2.9 ± 0.3 mM, respectively, p < 0.01). The same trend was found for the neuroglycopenic symptom scores, though not statistically significant (2.5 ± 0.3 vs 3.3 ± 0.2 mM, respectively, p = 0.06). The threshold for awareness occurred at 2.3 ± 0.3 vs 3.1 ± 0.2 mM, respectively (p = 0.01) (Table III).

**Cognitive function test** – On the entire population, the accuracy and time 4RT thresholds were 3.2 ± 0.2 and 2.7 ± 0.2 mM, respectively (p < 0.01). These two thresholds poorly correlated with HbA1c, before (r = 0.15, NS and r = 0.21, NS, respectively) and after adjustment (r = 0.11, NS and r = 0.18, NS, respectively). Taken as the major independent variable, diabetes duration weakly influenced the cognitive accuracy, before (r = 0.41, p = 0.08), and after adjustment (r = 0.39, p = 0.09), but not 4RT-time. Accuracy and time of responses for 4RT occurred at lower levels in the good glycaemic control group than in those with fair glycaemic control, but the differences were not significant (3.0 ± 0.3 vs 3.5 ± 0.2 mM, p = 0.1, and 2.6 ± 0.2 vs 2.8 ± 0.3 mM, p = 0.5, respectively) (Table III).

**DISCUSSION**

In agreement with previous reports, we have found that type 1 diabetic patients with strict glycaemic control required a more profound hypoglycaemic stimulus to trigger adrenaline response and warning symptoms, and to perceive hypoglycaemia, than those with fairly controlled diabetes [6-9, 12]. As expected and as a possible consequence of these abnormalities,
tightly controlled patients had a greater history of hypoglycaemic coma and unawareness. On the whole population, these thresholds correlated very well with glycaemic control before and after adjustment for confounding variables, i.e., age and diabetes duration, thus confirming the above results.

An unresolved question is whether a similar downshift occurs with the hypoglycaemic threshold for cognitive impairment ("adaptation"), or remains "fixed" in strictly controlled patients ("maladaptation" since early cognitive dysfunction increases the risk of severe hypoglycaemia and is indicative of no change in brain cell exposure to hypoglycaemic injury) [13, 19-23]. Using only 4RT, one of the most sensitive tests to evaluate cortical complex functions (especially vigilance and attention) [22], we have found that cognitive thresholds shift, but not significantly, and less than other thresholds in our patients with tight glycaemic control. i.e. cognitive function deteriorates earlier than symptoms and warning of hypoglycaemia, further worsening the risk of inappropriate behaviour and patient management of hypoglycaemia. This finding was further supported by the evidence that in our whole population, HbA1c values correlated poorly with accuracy and time of responses to 4RT (the studies may be underpowered to detect a small difference in cognitive thresholds). Our results are in agreement with some data from literature [27-30], but not all [24-26]. These discrepancies may lay in methodological differences [22], variability of cognitive thresholds in the normals [23], and relatively small number of subjects and heterogeneous population in most studies including ours [21, 39]. Also, the apparently paradoxical non-parallel shift of cognitive and neuroglucopenic thresholds, though both are originating from the cortex, may be due to regional variations of (mal) adaptation of cerebral tissues to tight glycaemic control [27].

There is a great controversy among investigators about which cognitive tests to use, how many and how often during the hypoglycaemic clamp [21, 39, 22]. We chose for 4-RT only, repeated every 30 minutes because it is a short (5 min.) and mechanical test, i.e., independent from the person who administers the test. A practice effect during hypoglycaemic clamp may have minimized the degree of cognitive dysfunction. However, absolute glycemic levels defined by 4-RT are of minimal importance since our major findings are based on relative values i.e., difference of glycemic thresholds between poorly and tightly controlled patients, and poor correlation with HbA1c. Conversely, batteries of complicated tests may increase arousal and reduce sensitivity [22].

Another important and barely explored aspect of hypoglycaemic cognitive dysfunction is the hierarchy of deterioration of the different types of intellectual functions and its possible deleterious effects over the daily life (e.g. driving). Some reports using 4RT-time [40-42], verbal fluency and other tests have shown that speed of reasoning is affected earlier than accuracy, suggesting that these subjects may be relatively protected against potential dangers (e.g. accidents). Other studies using 4RT only, either found a trend to similar glycemic levels for deterioration of the two cognitive functions, or that accuracy deteriorated only a little earlier than speed [10, 27]. In our study, using 4RT, we found that accuracy deteriorated significantly earlier than speed. This means that accuracy was sacrificed for speed, a mechanism which may contribute to higher risk of severe hypoglycaemia. However, the precision of responses showed more important inter-individual variability than those of speed, an effect possibly dependent on the heterogeneity of our population. Several experimental aspects may contribute to discrepancies between the studies. First, thresholds sometimes were defined based on different coefficient of variation [10, 27, 43]. To increase the confidence in our results, we used two cognitive dysfunction thresholds definitions, finding identical results. Second, some works only displayed one threshold for 4RT without clarifying which one [44]. Third, it is quite possible that the way to explain to each subject the major goal of this test (i.e., concentrate on speed or precision), and the time spent for training may induce a bias. Finally, our hypoglycaemic clamp was shorter than in other studies, thus allowing us to hypothesise that the faster rate of glucose fall may contribute to earlier deterioration of the most delicate and complex cognitive functions expressed, e.g. precision in reasoning. In the same way, duration of hypoglycaemia may influence cognitive results since, contrary to Amiel [45] and our data, Kerr [46] showed, using 4RT, that symptoms and cognitive function returned to basal values during a 2 h hypoglycaemic clamp despite persistent release of counterregulatory hormones. Moreover, Gold [47] found an improvement using the same test, at the end of 60 min of hypoglycaemia when compared to the beginning. These last two surprising findings may be explained by a partial adaptation or a practice effect [21, 22]. The effect of the rate of glucose fall and the duration of hypoglycaemia upon intellectual performances obviously deserves further investigation.

In conclusion, we have found only a modest adaptation of hypoglycaemic thresholds for cognitive impairment in type 1 diabetic patients with strict glycaemic control. Furthermore, we have observed that precision deteriorated earlier than speed of responses to visual stimuli. These "maladapted" reactions may contribute to the higher risk for severe hypoglycaemia in subjects with tight control. The major reason for the discrepancies and variable results between published studies including ours may be due to differences in methodological procedures, indicating the urgent need of an international consensus on these aspects.
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REFERENCES


12 Cryer PE. Hypoglycaemia begets hypoglycaemia in IDDM. Diabetes, 1993, 42, 1651-1663.


