Prevention of Hypoglycemia Using Risk Assessment With a Continuous Glucose Monitoring System

Carine Choleau,1 Petr Dokladal,2 Jean-Claude Klein,2 W. Kenneth Ward,3 George S. Wilson,4 and Gérard Reach1

Detection of hypoglycemia is considered to be a major advance that can be expected from a continuous glucose monitoring system (1–4). However, it may be preferable to orient the general goal of the system toward prevention, rather than detection, of hypoglycemia. First, this is what the patients are waiting for (5). Second, the occurrence of hypoglycemia has per se harmful metabolic effects, inducing insulin resistance (6) and increasing the risk of future severe hypoglycemia (7).

A hypoglycemia prevention system should generate in a timely fashion an alarm indicating that intake of sugar is necessary, i.e., taking into account the time necessary for sugar intestinal absorption. Rather than hypoglycemia itself, it should therefore detect a risk for hypoglycemia, defined as the moment when it estimates that the blood glucose concentration is expected to decrease below a hypoglycemic value (e.g., 70 mg/dl) within a given time (e.g., 20 min).

The aim of this experimental study, performed in rats, was to validate this concept. First, the detection of a hypoglycemia risk, as defined above, was achieved on the basis of serial determinations of blood glucose concentration. We observed that administering glucose 20 min before the expected occurrence of hypoglycemia prevented its occurrence. Second, we used a continuous glucose monitoring system consisting of a subcutaneous glucose sensor and an electronic control unit (8,9) in which we implemented a new software that was able to trigger an alarm based on the detection of such a hypoglycemia risk. In this article, we demonstrate that, despite that the system monitored glucose concentration in interstitial fluid and not in blood, this approach made it possible to administer glucose in an anticipatory way, leading to full prevention of hypoglycemia without overshoot hyperglycemia.

RESEARCH DESIGN AND METHODS

Experimental model and groups of animals. We first set up a model of controlled hypoglycemia in nondiabetic rats. Male fasted Wistar rats were anesthetized with halothane and instrumented with a 10-cm sterile silicone catheter indwelled into the right jugular vein and connected to a double catheter. After 1 h of recovery from surgery, rats were infused with 30% glucose at 27 mg·kg⁻¹·min⁻¹ and with insulin (Actrapid, Novo) at 0.2 units·kg⁻¹·h⁻¹, clamping the blood glucose concentration at 200 mg/dl. This infusion rate was found in preliminary experiments to increase plasma insulin from 1 to 5 ng/ml (data not shown). Seventy minutes after the beginning of infusions, glucose infusion was either stopped, or progressively decreased, in order to produce a steep, or shallow, decrease in blood glucose concentration, respectively. This would simulate the occurrence of hypoglycemia in patients treated with an insulin infusion pump. Rates of decrease in blood glucose concentration are given in Table 1.

For each of these two rates of decrease in glucose concentration, five groups of animals (five to six rats in all groups) were investigated. In group A, there was no attempt to prevent or correct hypoglycemia. In groups B and C, glucose was given when blood glucose concentration either reached 70 mg/dl (group B) or when it was estimated that it would reach this value within 20 min (group C). In these groups of rats, the detection of hypoglycemia (group B) or the recognition of the hypoglycemia risk (group C) was achieved by serial blood glucose sampling. In groups D and E, the same glucose load was administered when the detection of hypoglycemia (group D) or the recogni-
tion of the hypoglycemia risk (group E) was achieved by a continuous glucose monitoring system.

In all rats, glucose was measured in blood taken at the tail vein by using a glucose meter (Glucotrend). In groups D and E, interstitial tissue glucose concentration was in addition determined by a miniaturized implanted subcutaneous glucose sensor, the preparation of which is described elsewhere (10). The sensor was implanted the day before in the interscapular subcutaneous tissue and was polarized overnight with a miniaturized 650 mV battery as previously described (8). The next morning, the rats were anesthetized with halothane and the sensor was connected to an electronic control unit (ECU), which applies potential, acquires and stores the sensor current, and performs data processing, i.e., filtering and transforming the sensor output into an estimation of glucose concentration through a calibration procedure. In these experiments, a one-point calibration procedure was performed 30 min after the beginning of the glucose-insulin infusion, using a fixed value of 3 nA for the background current Io (9,11). The 3-nA value was chosen for Io because sensors prepared without active enzyme gave a similar background current (data not shown). We used a median filter on 11 samples (one point per 30 s), introducing a 2.5-min lag.

In all groups of animals, the intervention to correct, or to prevent, hypoglycemia used an intravenous glucose infusion simulating glucose appearance in blood from the gastric load. The profile of this glucose infusion was determined in a preliminary set of experiments performed in eight rats: blood glucose concentration was clamped at 220 mg/dl with glucose and insulin infusions. A 480-mg (1.6 ml of 30% glucose) glucose load was administered through a gastric catheter (Biotrol, Paris) 70 min after the beginning of the infusion (Fig. 1A). The decrease in the glucose infusion rate necessary to keep the blood glucose clamped at 220 mg/dl (Fig. 1B) allowed determination of the

FIG. 1. Intravenous glucose infusion simulating glucose intake from a 480 mg glucose gastric load. A: Blood glucose concentration (mean ± SE, n = 8). B: Glucose infusion rate necessary to clamp blood glucose concentration and determination of the appearance of glucose in blood from the gastric load. C: Semicontinuous intravenous profile simulating the glucose appearance in blood from the gastric load.
appearance of glucose in blood from the gastric load. This was used to design a semicontinuous profile of glucose appearance in blood from the gastric load (Fig. 1C). In all the subsequent experiments of this study, the same profile, referred to below as the intravenous glucose load, was used to correct or to prevent hypoglycemia, except that 240 mg and not 480 mg of glucose were used. (Preliminary studies, not shown, indicated that the 480-mg glucose load produced overshoot hyperglycemia.) The effect of the intragastric glucose load, determined here at 220 mg/dl in order to make it possible to observe a significant decrease in the glucose infusion rate, may be different at lower blood glucose concentrations (12).

Detection of hypoglycemia. Hypoglycemia was detected either on the basis of blood glucose concentration, measured every 5 min (groups B), or on the estimation by the sensor of subcutaneous, interstitial glucose concentration, which was determined every 30 s and triggered an alarm when it was <70 mg/dl (groups D).

Recognition of hypoglycemia risk. The recognition of hypoglycemia risk was performed in real time from the serial determinations of blood or interstitial glucose concentration. For the groups of rats in which this was based on the determination of blood glucose concentration (groups C), three consecutive values of blood glucose determined at 5-min intervals were used. The intravenous glucose load was started when it was determined that the time-related glucose slope would cross the 70-mg/dl hypoglycemic threshold within 20 min. This slope was computed in real time by plotting the data in Excel software using first order linear regression. For the groups in which this was based on the estimation of glucose concentration by the glucose sensor (groups E), this was achieved in real time by the ECU which analyzed 20 consecutive values sampled at 30-s intervals. Here also, the ECU triggered the alarm when the software estimated from the slope of the time-dependent estimation of glucose concentration by the sensor that this estimation would be <70 mg/dl within 20 min.

Data analysis. All data in text and figures are given as means ± SE, and the statistical significance was assessed by the paired Student’s t test and ANOVA.

RESULTS

Control groups. Eleven animals were administered a glucose-insulin infusion to clamp glucose concentration at ~200 mg/dl. The interruption of glucose infusion in group A1 (Fig. 2A) produced a steep decrease in blood glucose concentration (Table 1), which reached 32 ± 2 mg/dl by the end of the experiment. In group A2 (Fig. 2B), a progressive decrease in glucose infusion resulted in a

<table>
<thead>
<tr>
<th>Groups (n)</th>
<th>Slope of glucose decrease (mg·dl⁻¹·min⁻¹)</th>
<th>Glycemia at the beginning of glucose load (mg/dl)</th>
<th>Lag between the beginning of the glucose load and that of the increase in glycemia (min)</th>
<th>Glucose lowest value (mg/dl)</th>
<th>Glucose highest value (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (6)</td>
<td>5.5 ± 0.3</td>
<td>67 ± 1</td>
<td>22 ± 1</td>
<td>32 ± 2</td>
<td>81 ± 6</td>
</tr>
<tr>
<td>A2 (5)</td>
<td>1.4 ± 0.2</td>
<td>69 ± 1</td>
<td>18 ± 3</td>
<td>65 ± 1</td>
<td>100 ± 7</td>
</tr>
<tr>
<td>B1 (6)</td>
<td>5.0 ± 0.3</td>
<td>159 ± 11</td>
<td>22 ± 1</td>
<td>98 ± 8*</td>
<td>127 ± 8</td>
</tr>
<tr>
<td>C1 (6)</td>
<td>5.5 ± 0.3</td>
<td>104 ± 3</td>
<td>16 ± 2</td>
<td>89 ± 2†</td>
<td>109 ± 2</td>
</tr>
<tr>
<td>C2 (6)</td>
<td>1.6 ± 0.1</td>
<td>58 ± 5</td>
<td>14 ± 3</td>
<td>52 ± 7</td>
<td>83 ± 4</td>
</tr>
<tr>
<td>D1 (5)</td>
<td>4.0 ± 0.1</td>
<td>67 ± 6</td>
<td>21 ± 2</td>
<td>57 ± 6</td>
<td>101 ± 6</td>
</tr>
<tr>
<td>E1 (6)</td>
<td>5.0 ± 0.3</td>
<td>122 ± 9</td>
<td>20 ± 3</td>
<td>88 ± 2‡</td>
<td>116 ± 11</td>
</tr>
<tr>
<td>E2 (6)</td>
<td>1.7 ± 0.1</td>
<td>112 ± 5</td>
<td>15 ± 2</td>
<td>95 ± 5*</td>
<td>116 ± 6</td>
</tr>
</tbody>
</table>

Data are means ± SE. For each group of animals, 1 and 2 refer to the steep and shallow slopes of glucose decrease, respectively. *P < 0.0001 vs. 70 mg/dl; †P < 0.002 vs. 70 mg/dl; ‡P < 0.005 vs. 70 mg/dl.
shallow decrease in blood glucose concentration (Table 1), which reached 51 ± 2 mg/dl by the end of the experiment.

**Attempt to correct hypoglycemia at the detection of hypoglycemia determined from blood glucose concentration.** In group B1, the 240-mg glucose load was administered when the blood glucose concentration was found to be <70 mg/dl. As shown in Fig. 3A, there was a 20-min delay between the beginning of the glucose load and the beginning of the increase in blood glucose concentration. Thus, despite this administration of glucose, the blood glucose level decreased further, reaching a nadir of 58 ± 4 mg/dl, and subsequently remained <78 ± 6 mg/dl despite the glucose load. However, this is an effect of representing the mean of different experiments. Indeed, the mean of the lowest value observed in each individual experiment was 53 ± 3 mg/dl, and the highest value during recovery was 81 ± 6 mg/dl (Table 1).

The same procedure was applied in the context of a shallow decrease in blood glucose concentration (group B2). Fig. 3B represents the mean value of blood glucose concentrations, which reached a nadir of 73 ± 3 mg/dl and subsequently remained <87 ± 8 mg/dl. Table 1 gives the mean of the lowest (65 ± 1 mg/dl) and of the highest (100 ± 7 mg/dl) values of glucose concentration observed during recovery in each individual experiment.

**Attempt to correct hypoglycemia on the recognition of a hypoglycemia risk from blood glucose concentration.** The hypoglycemia risk was detected when it was estimated from serial measurements in blood that the blood glucose concentration would reach 70 mg/dl within 20 min. In group C1, in which the decrease in blood glucose concentration was steep (Fig. 4A), this phenomenon occurred for a blood glucose concentration of 159 ± 11 mg/dl. Despite the same 20-min lag between the start of the glucose load and the increase in blood glucose level (Table 1), hypoglycemia was completely prevented and blood glucose level remained >98 ± 8 mg/dl (Table 1). There was no subsequent overshoot hyperglycemia (the maximal blood glucose level was 127 ± 8 mg/dl).

In group C2, the same procedure was applied in the context of a shallow decrease in blood glucose concentration. As shown in Fig. 4B, hypoglycemia was also completely prevented. Table 1 gives the mean of the lowest and the highest values of glucose concentration observed in each individual experiment.

**Attempt to correct hypoglycemia at the detection of hypoglycemia determined from interstitial glucose concentration.** In group D1 (Fig. 5A), during the steep decrease in blood glucose concentration, the decrease in the estimation of glucose concentration by the sensor was delayed by ~5 min. Due to this delay, blood glucose concentration was already 61 ± 5 mg/dl when the 70-mg/dl threshold was detected by the subcutaneous sensor. The system triggered an alarm and the glucose load was started. Due to the lag between the start of the glucose load and the increase in blood glucose level, it was obviously too late to prevent hypoglycemia (even when estimated in blood). As shown in Table 1, the mean of the lowest value of blood glucose concentration reached in each individual experiment was 52 ± 7 mg/dl.

In group D2 (Fig. 5B), the same experiment was repeated with the shallow decrease in blood glucose concentration. In this group of animals, the decrease in the estimation of subcutaneous glucose concentration was variable. It preceded in three and followed in two experiments the decrease in blood glucose concentration, explaining the large SE bars. Therefore, when the estimation of subcutaneous glucose concentration reached 70 mg/dl, as a mean, blood glucose concentration was still 77 ± 6 mg/dl. Due to the lag between the start of the glucose load and the increase in blood glucose level, blood glucose concentration decreased further to 73 ± 4 mg/dl and, following the effect of glucose administration, it remained <80 ± 10 mg/dl. However, once again, these are values obtained by calculating the mean of the five experiments. The mean of the lowest values observed in each individual experiment was actually 57 ± 6 mg/dl (Table 1).

**Attempt to correct hypoglycemia on the recognition of a hypoglycemia risk from interstitial glucose concentration.** In group E1 (Fig. 6A), as in group D1 (Fig. 5A), during the steep decrease in blood glucose concentration, the decrease in the estimation of glucose in interstitial liquid followed that in blood with a lag of a few minutes. Despite this delay, the intravenous glucose prevented hypoglycemia, the mean of the lowest observed blood glucose concentration being 88 ± 2 mg/dl (Table 1). No overshoot hyperglycemia was observed (the maximal blood glucose concentration was 116 ± 11 mg/dl). This complete prevention of hypoglycemia was obtained despite the lag between the start of the glucose load and the increase in blood glucose level and was due to the anticipatory administration of glucose, achieved when the blood glucose concentration was still 122 ± 9 mg/dl (Table 1).

In the group E2, in which the decrease in blood glucose concentration was shallow, the decrease in the estimation of interstitial glucose concentration preceded by ~10 min the decrease in blood glucose concentration (Fig. 6B). Here again, despite the lag between the start of the glucose load and the increase in blood glucose level, hypoglycemia was completely prevented by the glucose load, performed when blood glucose concentration was still 112 ± 5 mg/dl. Once more, no overshoot hyperglycemia was observed, as the blood glucose concentration remained between 95 ± 5 and 116 ± 6 mg/dl (Table 1).

Table 1 summarizes these data. Despite the lag between the beginning of the load and that of the increase in blood glucose concentration, which was in all experiments 15–20 min (no statistical difference between groups, F = 2.039, P = 0.076), hypoglycemia was fully prevented without overshoot hyperglycemia in the groups of rats in which the glucose load was started when the hypoglycemia risk was detected (groups C1, C2, E1, and E2). This was, of course, not the case when the same glucose load was infused at the detection of hypoglycemia (groups B1, B2, D1, and D2).

**DISCUSSION**

In the past few years, several systems for continuous glucose monitoring have become available. The Continuous Glucose Monitoring System (CGMS), developed by MiniMed, uses a subcutaneous glucose sensor that provides the data at the completion of a 3-day recording.
Therefore, it cannot be used to detect on-line hypoglycemia, but only to estimate a posteriori the frequency and the duration of hypoglycemic episodes (13). Another system, commercially available (GlucoWatch), based on iontophoresis detection of glucose concentration in subcutaneous tissue, provides on-line monitoring of glucose concentration. The ability of this system to function as a hypoglycemic alarm has not been evaluated. However, a study using an a posteriori analysis of data indicated that in order to increase the sensitivity and the specificity of hypoglycemia detection, it was necessary to increase the threshold defining hypoglycemia (2). A new system, based

---

**FIG. 2.** Blood glucose concentration for the control groups. A: Steep decrease in blood glucose concentration (group A1, n = 6, mean ± SE). B: Shallow decrease in blood glucose concentration (group A2, n = 6).
on microdialysis (GlucoDay), gives an accurate estimation of glucose concentration in the low glucose range (3), but its ability to detect and prevent hypoglycemia has not yet been evaluated.

Indeed, the design of a hypoglycemic alarm using a subcutaneous glucose sensor is not straightforward. The fact that glucose is not monitored directly in blood, but in interstitial liquid, introduces a difficulty linked to discrepancies between changes in blood glucose concentration and the glucose level monitored in the interstitial tissue. For example, during a decrease in blood glucose concentration following administration of insulin in rats (14), or

FIG. 3. Effect of the intravenous glucose load started at the detection of hypoglycemia based on blood glucose determination. A: Steep decrease in blood glucose concentration (group B1, n = 6). B: Shallow decrease in blood glucose concentration (group B2, n = 5). The arrow refers to the time of hypoglycemia detection and the beginning of the glucose load.
FIG. 4. Effect of the intravenous glucose load started at the recognition of the hypoglycemia risk, based on blood glucose determination. A: Steep decrease in blood glucose concentration (group C1, n = 6). B: Shallow decrease in blood glucose concentration (group C2, n = 5). The arrow refers to the time of hypoglycemia risk recognition and the beginning of the glucose load.
in humans (15), the decrease in the subcutaneous glucose concentration was found to precede that in blood. However, this phenomenon is not always observed (15) and may depend on the rapidity of the decrease in blood glucose concentration (16): if this decrease is rapid, the decrease in interstitial glucose concentration will follow,
FIG. 6. Effect of the intravenous glucose load started at the recognition of hypoglycemia risk, based on interstitial blood glucose concentration. 
A: Steep decrease in blood glucose concentration (group E1, n = 6). B: Shallow decrease in blood glucose concentration (group E2, n = 6). ○, blood glucose concentration (mean ± SE). Continuous line: estimation of glucose concentration by the subcutaneous sensor calibrated at T60 min (mean ± SE). The arrow refers to the time of hypoglycemia risk recognition and the beginning of the glucose load.
PREVENTION OF HYPOGLYCEMIA

and not precede, that in blood (8). Furthermore, Moberg et al. (17) observed that in nondiabetic patients, following an insulin injection, interstitial glucose concentration remained low during recovery from hypoglycemia. The same observation was made during recovery from a hypoglycemic clamp performed in nondiabetic subjects (18). Taken together, these ill-defined discrepancies between blood and interstitial glucose concentration, which are the mere consequences of the complex physiology of interstitial glucose concentration (8), introduce a major difficulty in the design of a hypoglycemia alarm system based on the indirect detection of glucose concentration, i.e., in interstitial fluid rather than in blood. First, to detect hypoglycemia, the system compares a hypoglycemia threshold defined in blood with an estimation of interstitial glucose concentration. Second, this estimation of glucose concentration in interstitial fluid depends on the quality of the calibration, which may be jeopardized if it is performed in the presence of a discrepancy between blood and interstitial glucose concentration (11). This difficulty applies to any subcutaneous sensor (1,11), microdialysis (3), iontophoresis-based (2) system and possibly to any potential noninvasive glucose monitoring systems (19).

The second difficulty in the development of a system aimed to prevent, not only detect, hypoglycemia is linked to that in any case, there will be a delay between carbohydrate intake and the subsequent increase in blood glucose concentration, which suggests that the alarm must be triggered in an anticipated way. This can only be done if the system detects a risk of hypoglycemia, instead of hypoglycemia itself.

These considerations prompted us to develop a hypoglycemia alarm system based on risk assessment and to determine, first in an experimental study performed in rats, whether this strategy can work when glucose concentration is estimated in subcutaneous tissue rather than in blood. Data presented herein can be summarized as follows (Table 1): With a steep decrease (~5 mg/dl/min), the occurrence of hypoglycemia was not prevented when the glucose load, administered intravenously according to a profile mimicking the glucose appearance in blood from a gastric load, was started when the hypoglycemia was detected. This was first observed when hypoglycemia was defined on the basis of glucose concentration determined directly in blood. More interestingly, due to the lag between the decrease in blood and in interstitial glucose concentrations, the results were even worse when hypoglycemia was defined on the basis of the determination of interstitial glucose concentration. By contrast, the use of the hypoglycemia risk concept proved to be efficient: the occurrence of hypoglycemia was fully prevented, regardless of whether this transition was detected on the basis of blood or interstitial glucose concentration. The same result was obtained in a second series of experiments using a shallow decrease in blood glucose concentration (~1.5 mg · dl⁻¹ · min⁻¹), which is closer to decreases in the blood glucose concentration commonly observed in diabetic patients.

Interestingly, in the first group of rats (steep decrease, Figs. 5A and 6A), the decrease in the subcutaneous glucose concentration estimated with the glucose sensor clearly followed that in blood. Therefore, the actual blood glucose concentration will be already lower than the interstitial glucose concentration when this latter parameter will have reached the hypoglycemia threshold used, defined as a blood glucose concentration. A risk-based alarm system is therefore mandatory. By contrast, with a shallow decrease in blood glucose concentration, it becomes possible to observe the influence of insulin on glucose transfer from interstitial tissue to the surrounding cells, which should produce a faster decrease in interstitial glucose concentration than in blood (8). Indeed in these groups of animals (Figs. 5B and 6B), there were experiments in which the decrease in the estimation of glucose concentration in subcutaneous tissue preceded that in blood. This was especially visible on data presented in Fig. 6B, although it is also possible that the underestimation of subcutaneous glucose concentration observed in this group of animals was the consequence of the fact that the calibration was performed when the blood glucose concentration was increasing and had not yet reached a plateau, as in the other groups of animals. Incidentally, this points out the influence of the discrepancy between blood and interstitial glucose concentration on the outcome of the sensor calibration (11).

In conclusion, this article provides the experimental validation of a new method of prevention of hypoglycemia using an alarm triggered by the detection of a hypoglycemia risk defined not by a glycemic threshold or a gradient, but by the time remaining before hypoglycemia is expected to occur. We showed that this strategy can be efficient even when the sensor measures glucose concentration in interstitial fluid, i.e., under ill-defined conditions. Whether these experimental data, obtained in rats, will be relevant to the use of continuous glucose monitoring in humans requires further investigation. Also, the experimental design used in this study simulated continuous insulin infusion at a fixed rate by a pump, and the situation may be different with changing plasma insulin levels resulting from a multiple insulin injection regimen. Nevertheless, we suggest that the time defining a hypoglycemia risk, as in this study, should be roughly identical to the delay between the carbohydrate intake and the beginning of recovery from hypoglycemia (~20 min, see Table 1). In addition, this parameter may be customized to each individual patient.

Finally, this method fully prevented hypoglycemia without overshoot hyperglycemia. It is tempting to speculate that this was due to the early characteristics of the intervention, occurring before the involvement of the counter-regulation mechanisms (6). This may represent another advantage of the strategy to prevent rather than correct hypoglycemia. In conclusion, this article proposes clearly, for the first time, the concept of a hypoglycemia prevention system in lieu of a hypoglycemia alarm which is capable of detecting hypoglycemia and thereby helping the patient to correct it, but provides no opportunity to avoid its occurrence.

ACKNOWLEDGMENTS

This work was supported by a grant from the Center for Disease Control R08/CCR017796 (W.K. Ward, principal investigator), NIH DK55297 (G.S. Wilson, principal investigator), and Aide aux Jeunes Diabétiques.
REFERENCES


