Continuous Glucose Monitoring in the Free-Moving Rat

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The aim of this work was to set up an experimental model of glycemic fluctuations for assessing in the conscious freely moving rat, the performance of a continuous glucose-monitoring system, using a pocket-calculator–size electronic control unit and a miniaturized subcutaneous glucose sensor. The well-known triphasic glycemic pattern following streptozotocin injection (initial peak and secondary hypoglycemia preceding the establishment of permanent hyperglycemia) was used as a way to obtain spontaneous changes in blood glucose level over a wide concentration range. This report demonstrates that streptozotocin injection produced highly reproducible changes in the current generated by the sensor: an initial peak and a secondary nadir, during which blood sampling provided the evidence of hyperglycemia associated with immunoreactive hypoinsulinemia, and of hypoglycemia associated with hyperinsulinemia, respectively. This reproducible experimental model should be valuable for the assessment of a continuous glucose-monitoring system.

TO PROVIDE continuous monitoring of blood glucose concentration for the management of diabetes therapy,[1,2] our laboratories have developed a miniaturized amperometric glucose sensor in the form of a wire,[3] which specifically measures glucose essentially independently of oxygen pressure,[4] and in which interferences by ascorbate and acetaminophen have been eliminated.[5-7] This sensor has been investigated by implantation in the subcutaneous tissue of rats,[8] dogs,[9,10] and human volunteers.[11] A wearable electronic control unit (ECU) has been developed to transduce, record, filter, and process the signal generated by the sensor.[12] The aim of this work was to set up a reproducible animal model of glycemic fluctuations to assess the performance of the continuous glucose-monitoring system under conditions simulating the changes in blood glucose concentration that can be observed in a diabetic patient.

The diabetogenic drug streptozotocin is known to produce in the rat an initial increase in blood glucose, followed by transient hypoglycemia before the establishment of permanent hyperglycemia 24 hours after injection. These triphasic changes in plasma glucose concentrations are due, respectively, to an initial inhibition of insulin secretion, a massive release of insulin, and finally a definitive insulin deficiency due to β-cell destruction.[13] We first set up a system for evaluating the glucose-monitoring system in the conscious freely moving rat over a period of several days. Second, we used the variations in blood glucose following streptozotocin injection as an experimental model to evaluate performance of our continuous glucose-monitoring system.

MATERIALS AND METHODS

Glucose Sensors

Preparation of the miniaturized glucose sensor has been described elsewhere.[3] The sensor consists of a platinum anode covered with Teflon, except for a 1.5-mm long cavity near its extremity, where glucose oxidase was layered. A silver/silver chloride cathode was wrapped around the Teflon coating. The sensor was then coated with polyurethane. The external diameter of the sensor was approximately 0.35 mm.

Wearable ECU

The glucose sensor was connected to a wearable, battery-driven (6-V) ECU (150 × 80 × 30 mm, 250 g; École des Mines, Fontainebleau, France), which controls the sensor-applied potential, and acquires, digitizes, and stores the sensor glucose-dependent current. The memory allows the storage of data for up to 6 days (one sample per minute). The ECU continuously displays the values of the current or of its transformation into a glucose concentration (discussed later) on a liquid crystal display (LCD). The digitized signal was processed in real time by an original gradient algorithm. A gradient filter is more efficient than classical filters of similar complexity, because it is simultaneously based on the properties of the signal to be measured (maximum admissible gradient) and on the noise characteristics (duration of perturbations). This smoothing procedure removes all spikes in which the gradient of the signal is greater than the maximum admissible value and which has a width narrower than the filter size. It decreases the amplitude of the spikes that have a width wider than the filter size. This gradient algorithm is described in the Appendix.

Surgery and Implantation

A 7-cm sterile silicone catheter (Medical Grade Silicone Tubing; 0.64 mm ID, 1.19 mm OD; Sigma Medical, Nanterre, France) was connected to a 21-gauge bent needle. A 1.5-cm connector (0.94 mm ID, 1.56 mm OD; Tygon, Norton, Vermon, OH) was placed between the tip of the needle and a polyethylene catheter (0.58 mm ID, 0.96 mm OD; Diagnostics Merck-Biotrol, France), which was indwelled through a metallic tether and was finally connected to a swivel (Harvard reference 568154; Biosciences, Ealing, France). Another piece of silicone catheter was connected to the swivel through a 21-gauge needle to a syringe containing 1% heparin (Panpharma, Luitré, France) in physiological saline solution. The anode and the cathode of a glucose sensor were attached through a connector to two wires running through the tether and attached to the bottom part of the swivel. Finally, the wires (anode and cathode) linked to the top part of the swivel were connected to the electronic control unit.

Seven male Wistar rats (250 to 300 g) were anesthetized with halothane. The silicone catheter was indwelled into a jugular vein, its tip being placed in the right atrium, and tunnelled through the subcutaneous tissue to the interscapular area. Then, the catheter was connected to the

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Submitted July 31, 1997; accepted January 15, 1998.

Supported by Institut National de la Santé et de la Recherche Médicale (grant CNAMTS), National Institutes of Health grant no. DK 30718, Aide aux Jeunes Diabétiques (grant to V.T.D.).

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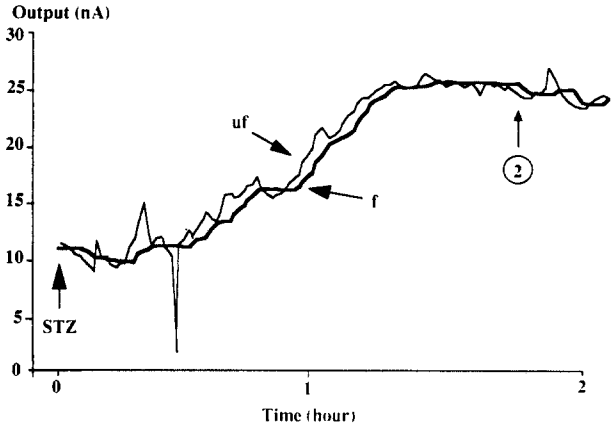

Fig 1. Individual experiment showing the unfiltered and the filtered signal after streptozotocin injection. The second blood sampling was performed when a stable plateau was observed.

Tygon catheter and filled with heparinized saline. The sensor was implanted in the dorsal subcutaneous tissue of the rat through a 20-gauge cannula, which was removed, leaving the sensor in place. The sensor was connected to the ECU through the swivel and the rat was placed in a single cage, under free-moving conditions, with food and water available ad libitum. The current generated by the sensor was displayed every 30 seconds on the LCD of the ECU and sensor output was acquired and stored every minute. The gradient filter was used to process the signal. A typical example of the filter efficiency is shown in Fig 1.

Injection of Streptozotocin and Blood Samples

The morning after the surgery, streptozotocin (Zanozar; Upjohn, Paris, La Défense, France; 50 mg/kg body weight) was injected through the jugular catheter. Four 2-mL blood samples were drawn on the basis of the changes in the current displayed by the ECU: immediately before streptozotocin injection, at the first peak of current, at the nadir of the current, and 24 hours after streptozotocin injection. The peak and nadir samples were obtained when the filtered current was stable (<0.03-nA variation) for at least 10 minutes. Samples were immediately centrifuged, and plasma was stored until glucose and insulin determination. The erythrocyte pellet was resuspended with saline solution and immediately reinjected into the rat. Glucose concentration was determined in 10 μL of plasma using a Beckman Analyzer (Fullerton, CA). Plasma immunoreactive insulin was measured with a radioimmunoassay kit using human insulin as standard (CEA, Gif-sur-Yvette, France), using an antibody that crossreacts with human and rat insulin. The lower limit of the assay was 15 pmol/L with a within- and between-assay coefficient of variation of 6%. All samplings were run in the same assay.

In Vivo Calibration of the Sensor, and Presentation of Results and Statistics

To transform the current into an estimation of glucose concentration, a two-point calibration procedure was used, taking into account blood glucose concentration and current at baseline and at the first hyperglycemic peak, respectively. In vivo sensitivity, S, and a theoretical current in the absence of glucose, Io, were calculated by linear extrapolation.[14] Then, at any time, the estimation of glucose concentration, G(t), in the subcutaneous tissue was calculated from the current, I(t), by the equation:

$$G(t) = [I(t) - I\hat{O}]/S.$$

All data in the text and figures are presented as the mean ± SEM. Statistical significance was assessed by paired Student's *t* tests.

RESULTS

The sensor output (Fig 2A) increased from 10.4 ± 1.5 (C1) to 25.0 ± 2.3 nA (C2, $P = .0001$ *v* C1), then decreased to 6.3 ± 1.7 nA (C3, $P = .001$ *v* C1), and finally increased to 23.0 ± 2.7 nA (C4, $P = .003$ *v* C1). On the basis of these changes in the current, displayed every 30 seconds on the ECU, four blood samples were drawn for glucose and insulin determination (Fig 2B): plasma glucose concentration increased from 7.7 ± 0.4 (G1) to 22.1 ± 1.2 mmol/L (G2, $P = .0001$ *v* G1), then decreased to 5.0 ± 0.8 mmol/L (G3, $P = .0007$ *v* G1), and finally increased to 23.5 ± 1.5 mmol/L (G4, $P = .0001$ *v* G1). The expected opposite trends in plasma immunoreactive insulin concentration were observed: a decrease from 151 ± 43 (I1) to 77 ± 13 pmol/L (I2, $P = .02$ *v* I1), then an increase to 329 ± 52 pmol/L (I3, $P = .03$ *v* I1), and finally a decrease to 73 ± 35 pmol/L (I4, $P = .08$ *v* I1).

Kinetics of changes in the current following the administration of streptozotocin are represented in Fig 3 and analyzed in Table 1, illustrating the reproducibility of both the magnitude and the timing of the observed events. The current began to increase 61 ± 4 minutes (d1) after streptozotocin injection from a basal level of 10.4 ± 1.5 nA. The duration of the increase was 64 ± 7 minutes (d2, 14.6 ± 1.9 nA increment). The current

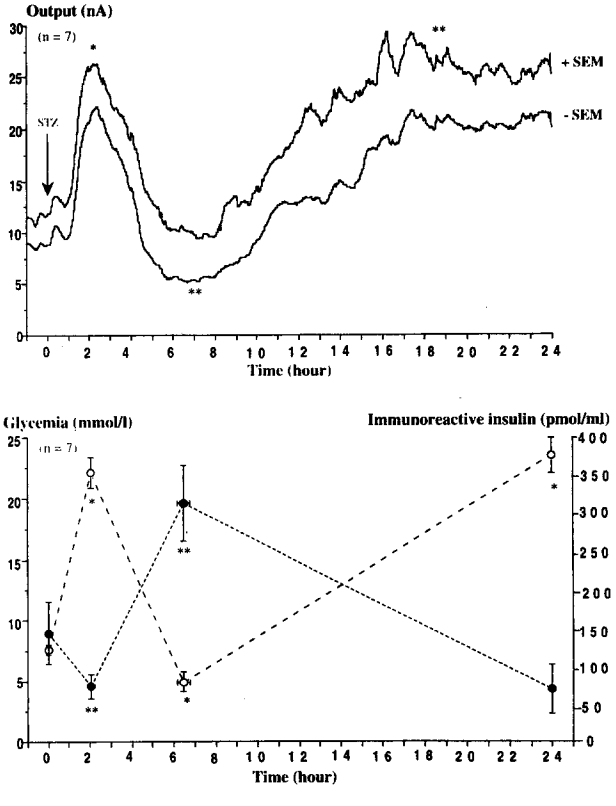

Fig 2. Average results of 7 experiments (mean ± SEM). (A) Filtered current; (B) plasma glucose (○) and insulin (●) concentration, mean ± SEM. Differences are significant: *$P < .0007$, **$P < .003$ *v* initial values.

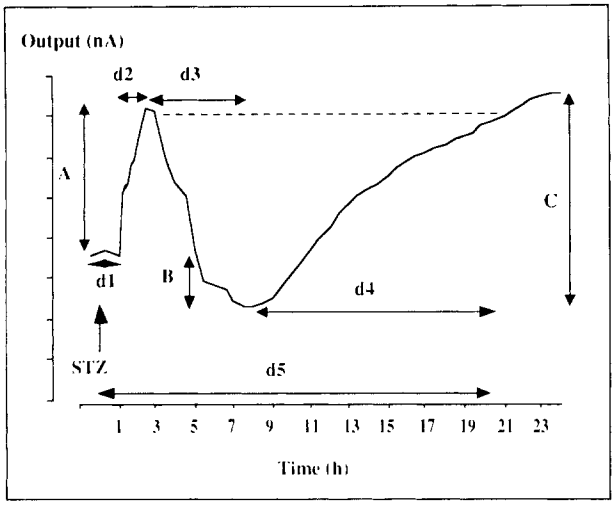

Fig 3. Sensor output profile following streptozotocin injection (see text for details and Table 1 for parameter analysis).

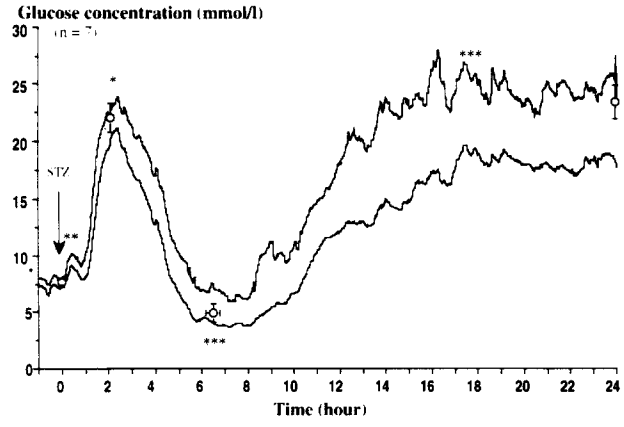

Fig 4. Continuous glucose monitoring (_) and glycemia (○) fluctuation during the 24 hours following administration of streptozotocin (STZ) in the free-moving fed rat (n = 7, mean ± SEM). STZ injection was followed by a transient peak in subcutaneous glucose concentration (P = .002 v initial value), observed 20 minutes after STZ injection. This peak preceded the major increase in subcutaneous glucose concentration (*P = .0001 v initial value). Subcutaneous glucose concentration continued to decrease to a nadir (***P = .01). The diabetic states is reached ≃17 hours after STZ injection (***).**

decreased subsequently within 264 ± 19 minutes (d3) to the nadir value (−4.1 ± 0.8 nA decrement from basal value). Finally, the second high value of current (25.0 ± 2.3 nA) was reached 398 ± 71 minutes later (d4), ie, 1,049 ± 137 minutes (17 hours 30 minutes, d5) after streptozotocin injection. Twenty-four hours after streptozotocin injection, the current was 23.0 ± 2.7 nA.

These values of current were transformed a posteriori into an estimation of subcutaneous glucose concentration using a two-point calibration procedure, based on values of the current and plasma glucose concentration at baseline and at the initial peak. Figure 4 shows continuous glucose monitoring during 24 hours following administration of streptozotocin in the free-moving fed rat. Values of plasma glucose concentrations of the four blood samples are also shown. Close examination of this figure yields three observations: (1) streptozotocin injection was immediately followed by a transient peak in blood glucose, from 7.7 ± 0.4 to 9.7 ± 0.5 mmol/L (P = .002), observed 20 minutes after streptozotocin injection. This peak preceded the major increase in subcutaneous glucose concentration, which started 1 hour after streptozotocin injection; (2) subcutaneous glucose concentration continued to decrease slightly after the third blood sample to a 4.8 ± 1.1 mmol/L nadir observed at 435 minutes; (3) the figure confirms that the diabetic state is reached approximately 17 hours after streptozotocin injection.

DISCUSSION

We set up an experimental model of glycemic fluctuations in the free-moving rat, in which frequent blood sampling and continuous glucose monitoring over a 24-hour period are possible, using a glucose sensor implanted in the subcutaneous tissue connected to an ECU able to continuously display the current on a LCD. A gradient filter was used to eliminate the noise due to movement of the rat. This model was validated by the demonstration that it is possible to monitor on a continuous basis the changes in blood glucose that follow streptozotocin injection in the rat. First, this study provides evidence that these changes are highly reproducible, not only concerning their amplitude, but also their timing (Table 1). Second, the use of this model confirmed that our continuous glucose-monitoring system is an efficient tool for detecting changes in plasma glucose level over a wide concentration range. Indeed, we were able to detect all of the known glycemic events that precede the

Table 1. Time Course of Changes in the Current, Plasma Glucose, and Insulin Concentration Following Streptozotocin Injection in the Free-Moving Fed Rat (N = 7)

Time (min)					Output's Variations (nA)		
d1	d2	d3	d4	d5	A	B	C
61 ± 4	64 ± 7	264 ± 19	398 ± 70	1,049 ± 137	14.6 ± 1.9	−4.1 ± 0.8	16.8 ± 3.3

	S1	S2	S3	S4
Time (min)	0	124 ± 7	388 ± 19	1,440
Output (nA)	10.4 ± 1.5	25.0 ± 2.3	6.3 ± 1.7	23.0 ± 2.7
Glycemia (mmol/L)	7.7 ± 0.4	22.1 ± 1.2	5.0 ± 0.8	23.5 ± 1.5
Insulinemia (pmol/L)	151 ± 43	77 ± 13	329 ± 52	73 ± 35

NOTE. Results are expressed as the mean ± SD. See Fig 3. S1 to S4 are the 4 samples following streptozotocin injection with their respective timing, the corresponding sensor output, glycemia, and immunoreactive plasma insulin concentration.

establishment of permanent, streptozotocin-induced, hyperglycemia in the rat. By taking only four blood samples on the basis of the sensor output, we were able to confirm the proposed mechanism for diabetes induction.

Streptozotocin is a diabetogenic drug that induces a triphasic pattern in blood glucose and plasma immunoreactive insulin concentration.[13] In these ad libitum–fed animals, the initial increase in blood glucose (Fig 2) was observed to be more important than previously described[13] and was similar to the value observed 24 hours after the drug injection. It was associated with a decrease in plasma insulin and may therefore represent a first step in the islet attack by the drug, leading to an inhibition of insulin secretion. This increase in blood glucose concentration started approximately 1 hour after administration of the drug, and the peak in glucose concentration was reached within 1 hour. Continuous glucose monitoring provided in addition the unique evidence that this peak in glucose concentration was actually preceded by a transient, slight (≈2 mmol/L magnitude), but significant ($P = .002$) increase in blood glucose. As plasma insulin was not determined during this period, its significance is unclear. It may represent a glycogenolytic effect of streptozotocin on the liver, one of the possible explanations proposed for the low glycogen liver content observed in rats 2 days after streptozotocin injection.[15] The lowest glucose concentration (≈5 mmol/L) was observed 7 hours after streptozotocin administration. One hour before, plasma glucose was lower than basal value and was paradoxically associated with a dramatic increase in plasma immunoreactive insulin concentration, which reached a level double the basal insulin concentration. Therefore, this hypoglycemic episode is likely due to a massive insulin release from destroyed β cells. It was followed by a progressive increase in the current, which reached the diabetic range 17 hours after administration of the drug. Twenty-four hours after streptozotocin administration, persistent hyperglycemia was associated with low plasma immunoreactive insulin concentrations. Therefore, this report provides, for the first time, a precise description of the timing of diabetes appearance in the streptozotocin-induced diabetic rat.

In this study, the transformation of sensor output into an estimation of glucose concentration using a two-point calibration procedure[14] was performed a posteriori, by taking into account the values of sensor output and plasma glucose concentration at baseline and at the first hyperglycemic peak. This calibration procedure was validated by the fact that the subsequent estimation of glucose concentration at the secondary sensor output nadir and 24 hours after streptoztocin injection fitted with the actual plasma glucose concentration (Fig 4). This calibration procedure can also be performed on line, since it is possible to enter into the ECU, during a temporary connection to a personal computer, the values of plasma glucose concentration and of the corresponding current, the electronic control unit calculating the calibration parameters S and Io and transforming subsequently the sensor output into an estimation of the glucose concentration, which can be displayed every 30 seconds on the LCD.[11]

In conclusion, this most reproducible animal model should be useful for further evaluation of our continuous glucose-monitoring system. Incidentally, we were able to sample blood glucose in a timely fashion to observe an inappropriate plasma insulin concentration concomitant with a low plasma glucose value. Such a system may therefore be useful not only in the management of diabetes, but also in the assessment of fasting hypoglycemia.

APPENDIX

The gradient algorithm works as follows: MG is the maximum gradient accepted for the variation of the glycemia, expressed in percentage, and N is the size of the filter, expressed in number of points. These N points consist of the value of current obtained during the last measurement and of the N-1 previous values of current. The filtering process extracts from these N points the value VAL, which presents the lowest absolute difference (LAD), with the last filtered value (LFV).

If the LAD value is less than or equal to MG × LFV/100, the new filtered value NFV is given by NFV = LFV + S × LAD/M, where S is the sign of the difference VAL − LFV and M is the number of points between LVF and VAL.

If the LAD value is greater than MG × LFV, then NFV = LFV + S × MG × LFV/100.

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