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Strategies for calibrating a subcutaneous glucose sensor*

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Summary

The calibration of the glucose sensor is a critical issue for the subcutaneous implantation of such devices, since it allows from the sensor output the estimation of the prevailing subcutaneous glucose concentration. This study has compared estimations of subcutaneous glucose concentration in rats, calculated from the sensor's signal by using calibration parameters (sensitivity coefficient and background current) determined either under *in vitro* or *in vivo* conditions. The *in vitro* calibration was performed in phosphate buffer at 37 °C. The *in vivo* parameters were calculated by comparing changes in the sensor output current to concomitant changes in blood glucose concentration, induced by intravenous insulin injection and glucose infusion. The apparent subcutaneous glucose concentration, calculated with *in vitro* parameters, remained much lower than the concomitant blood glucose levels throughout the experiments. By contrast, when it was calculated with the calibration parameters determined *in vivo*, either in the hypoglycemia or hyperglycemia range, the apparent subcutaneous glucose concentration well reflected blood glucose levels with a five min time lag. We conclude that it is the two-point *in vivo* calibration procedure which allows the calculation of an apparent subcutaneous glucose concentration best reflecting blood glucose levels.

Introduction

The calibration of the glucose sensor is a critical issue for its subcutaneous implantation, allowing the calculation of the prevailing subcutaneous glucose concentration from the sensor output. All calibration procedures are based on the comparison of two points of glucose concentration with their concomitant points of sensor output. This makes it possible to calculate for each experiment both a sensitivity coefficient, expressed as the ratio between the changes in sensor output and those in glucose concentration ($\Delta I/\Delta C$), and the background current, i.e., the signal corresponding to 0 mmol/l glucose (Fig. 1A). Three strategies can be used to calibrate a subcutaneously implanted glucose sensor: *in vitro*, one-point *in vitro*/one-point *in vivo*, and two-point *in vivo* calibrations. Indeed, the calibration could be based on *in vitro* values of these two parameters. This assumes that both the current observed in the absence of glucose (I_0) and the sensitivity of the sensor are identical under *in vitro* and *in vivo*

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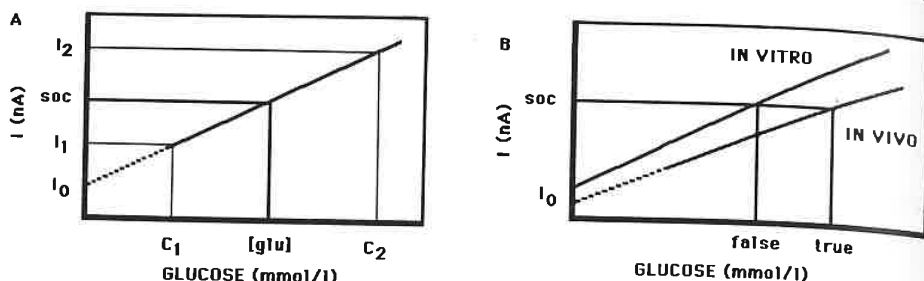


Fig. 1A) Scheme of a calibration procedure based on the comparison of two points of glucose concentration (C_1 and C_2) with the concomitant points of sensor output (I_1 and I_2). This comparison makes it possible to calculate the calibration parameters: the sensitivity coefficient, expressed in $\text{nA}/\text{mmol} \cdot \text{l}^{-1}$ glucose, and the background current (I_0), i.e., signal corresponding to 0 mmol/l glucose. These two parameters allow for the calculation of a glucose concentration $[\text{glu}]$, from the sensor output current (soc). B) In vivo calibration based on the in vitro and in vivo values of the calibration parameters. If these values are not identical, the in vivo calibration based on in vitro parameters would result in the miscalculation, from the sensor output current (soc), of the subcutaneous glucose concentration

conditions. This, however, might not be so, and the calculation of a subcutaneous glucose concentration from the sensor signal would therefore be false (Fig. 1 B). A second possibility in calibrating the sensor would be to adjust the initial sensor output to the concomitant blood glucose concentration. However, this is a one-point in vitro/one-point in vivo calibration procedure, since it assumes that the in vitro and the in vivo values of I_0 are identical and relatively small, compared to the sensitivity coefficient. To avoid these assumptions, an in vivo two-point calibration procedure can be used. We have proposed such a method which makes it possible to transform the recorded current into an estimation of the subcutaneous glucose concentration without taking into account the in vitro data [1].

The aim of this work was, firstly, to compare the estimated subcutaneous glucose concentration calculated from the sensor signal by means of in vitro or in vivo calibration parameters; secondly, to compare the apparent subcutaneous glucose concentration obtained with the in vivo two-point calibration performed in low and high levels of glucose concentration. These calculations were performed during variations of blood glucose concentration induced by intravenous insulin injections or glucose infusions in anaesthetized rats.

Materials and methods

The needle type glucose sensor

The amperometric needle-type glucose sensor has been described elsewhere [1]. Briefly, it consists of a silver or silver chloride coated steel needle (cathode) containing an isolated platinum wire (anode), the tip of which is covered with glucose-oxidase entrapped in cellulose acetate and reticulated with glutaraldehyde, and covered thereafter with a polyurethane layer. Its external diameter measures 1.2 mm.

In vitro calibration

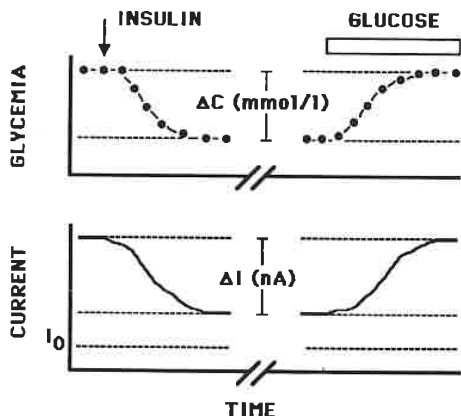
The sensors were connected to an amperometric unit (PRG-Del, Solea Tacussel Electronique, Villeurbanne, France) applying a 650 mV voltage difference between the anode and the cathode, thus allowing for current measurement, expressed in nanoamperes and recorded by a two-channel pen-recorder (SE 120, Goerz Electro,

Vienna, Austria). The *in vitro* characteristics of the sensors, i.e. the sensitivity, expressed in nA/mmol/l glucose, the linear range of the calibration curve, and the response time ($t_{90\%}$), were determined, before implantation and after explantation, in a thermostated (35–37 °C), stirred, buffered solution (0.15 mol/l phosphate, 0.15 mol/l NaCl, pH 7.4), during stepwise increases of the glucose concentration from 0 to 30 mmol/l.

In vivo calibration

Overnight fasted male Wistar rats ($n = 7$, 250–300 g body weight, Janvier, Saint Berthevi, France) were anaesthetized with pentobarbital (50 mg/kg intraperitoneally, Clin Midy, Saint Jean de la Ruelle, France), and polyethylene (Biotrol 3, Biotrol, France) and silicone (Silastic 602-135, Dow Corning, Midland Mich, U.S.A.) catheters were inserted, respectively, into the left jugular vein and homolateral carotid artery, for glucose or insulin injection and for blood sampling for the determination of the blood glucose concentration. After *in vitro* characterization, the sensor was implanted in the subcutaneous tissue through a small cervical incision. Animals were kept spontaneously breathing under a flow of carbogen (O₂–CO₂, 95:5) and warmed under a lamp, to prevent peripheral vasoconstriction.

At least 1 h after surgery, when blood glucose and the sensor output were stable, 1 U of insulin (Endopancrine U40, Organon, Saint Denis, France) was injected through the jugular catheter. When the insulin-induced fall in blood glucose reached a plateau, a continuous intravenous glucose infusion (20 to 60 mg/kg · min, infusion pump Infu 362, Datex, Uhwiesen, Switzerland) was initiated in order to bring blood and subcutaneous glucose to hyperglycemic levels. Blood samples (0.3 ml) were serially drawn through the carotid catheter, at 5-min intervals, from –10 to 50 min, for the insulin induced hyperglycemia, and from 0 to 40 min for the glucose infusion. Samples were immediately centrifuged and plasma glucose concentration was determined with a glucose analyzer (Beckman, Fullerton Ca., U.S.A.). An *in vivo* method for sensor calibration was used to calculate the apparent subcutaneous glucose level [1]. Briefly, following the insulin injection, the sensor output decreased to reach a plateau, as did the blood glucose concentration (Fig. 2).



$$\text{S.C.} = \Delta I / \Delta C \quad \text{A.S.G.C.} = (I - I_0) / \text{S.C.}$$

Fig. 2. *In vivo* two-point calibration.

Following insulin injection or glucose infusion, blood concentration and the sensor output reach a new plateau. The pre-insulin or glucose and post-insulin or glucose steady states of the blood glucose level and of the sensor output are used to calculate an *in vivo* sensitivity coefficient (S.C.), expressed in nA/mmol · l⁻¹, as the ratio between the change in the sensor current and the change in glucose concentration, and an extrapolated *in vivo* residual current (I_0) which would be observed in the absence of glucose. The calculated apparent subcutaneous glucose concentration (A.S.G.C.) is obtained by subtracting from the sensor current observed at a given time (I), the *in vivo* background current (I_0), and then by dividing the resultant current by the *in vivo* sensitivity coefficient (S.C.).

The pre-insulin and post-insulin steady states of the blood glucose level and of the sensor output made it possible to calculate by a linear regression function the *in vivo* sensitivity coefficient, expressed in nA/mmol/l, and the *in vivo* background current, i.e., the extrapolated signal corresponding to 0 mmol/l glucose (I_0). These parameters were also calculated taking into account the pre-glucose and post-glucose plateaus. The estimation of the subcutaneous glucose levels was obtained by subtracting from the sensor current, observed at a given time, the *in vivo* background current (I_0), and by dividing the resultant current by the sensitivity coefficient.

Expression of results and statistics

All data are given as mean $\pm$ SEM, and their statistical significance was assessed by the two-tail paired *t*-test.

Results

Sensor calibration

The *in vitro* characteristics and calibration parameters of the sensors, determined before implantation and after explantation, are summarized on Tab. 1. Following intravenous injection of 1 U insulin, blood glucose levels decreased from 5.9 ± 0.2 to a 2.4 ± 0.3 mmol/l plateau at 50 min and the current generated by the sensor decreased, with a 5 min time lag, from 11.54 ± 1.11 to a 5.15 ± 0.54 nA plateau ($n = 7$; $p < 0.001$). An intravenous glucose

Table 1

In vitro and *in vivo* characteristics and calibration parameters of the glucose sensor

	Before <i>In vivo</i>	After <i>In vivo</i>
<i>In vitro</i>		
$\Delta I/\Delta C$ (nA/mmol $\cdot$ l $^{-1}$)	2.50 ± 0.48	2.17 ± 0.20
I_0 (nA)	4.37 ± 1.07	2.94 ± 0.65
Linearity (mmol/l)	18.0 ± 1.3	18.0 ± 2.2
$t_{90\%}$ (s)	41 ± 4	43 ± 4
<i>In vivo</i>	Calibration 1	Calibration 2
$\Delta I/\Delta C$ (nA/mmol $\cdot$ l $^{-1}$)	1.67 ± 0.26	1.76 ± 0.28
I_0 (nA)	1.00 ± 0.80	1.03 ± 0.85

The sensors were characterized *in vitro* in a thermostated (35–37 °C), stirred, buffered solution (0.15 mol/l phosphate, 0.15 mol/l NaCl, pH 7.4). The sensitivity coefficients are expressed as $\Delta I/\Delta C$; the background currents are given by I_0 ; linearity is given as the upper limit of the linearity range of the calibration curve; the response time is given as $t_{90\%}$. *In vivo* calibration 1 and calibration 2 parameters were calculated, respectively, during insulin injection and glucose infusion ($n = 7$, mean $\pm$ SEM)

infusion was started at 55 min. Blood glucose increased progressively from 2.5 ± 0.3 to 11.3 ± 0.3 mmol/l at 95 min followed, with a 5 min time lag, by the sensor current which rose from 5.17 ± 0.54 nA to 19.95 ± 2.35 nA ($p < 0.001$). The *in vivo* calibration parameters, determined by taking into account the values of blood glucose concentration and sensor output obtained before and after insulin injection (calibration 1) and glucose infusion (calibration 2), are summarized in Tab. 1. Both calibration parameters were well correlated (sensitivity coefficients: $r = 0.988$, Fig. 3A; background currents: $r = 0.980$, Fig. 3B). By contrast, the *in vitro* and *in vivo* calibration parameters were poorly correlated:

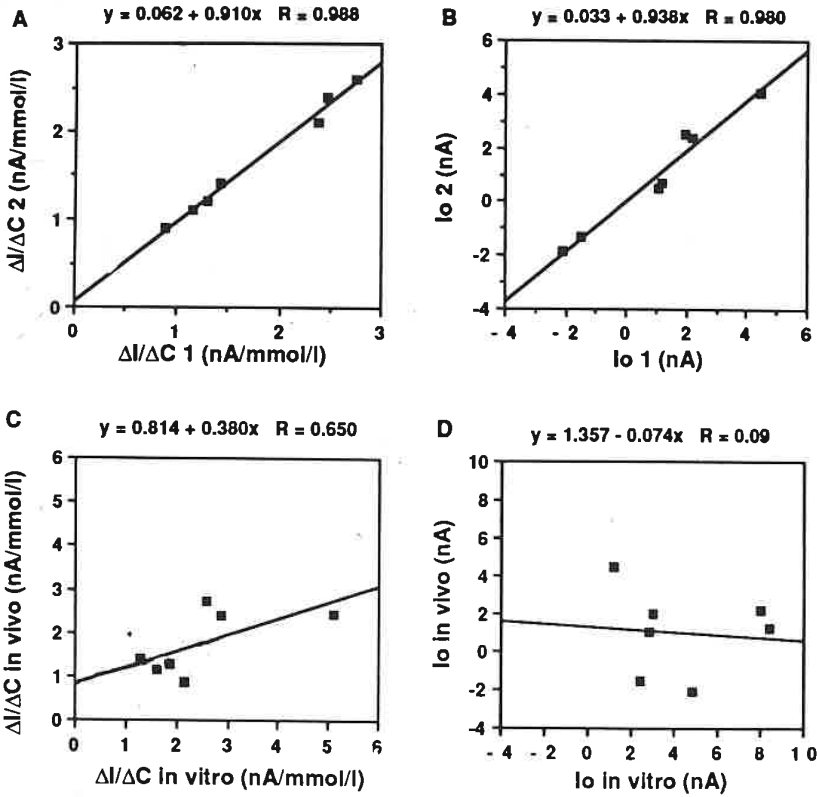


Fig. 3. Correlations between the values of the calibration parameters determined for each experiment ($n = 7$). A) sensitivity coefficients ($\Delta I/\Delta C$) and B) background currents (I_0) determined during insulin induced hypoglycemia (Calibration 1) and glucose infusion (Calibration 2). C) sensitivity coefficients ($\Delta I/\Delta C$) and D) background currents (I_0) determined before implantation (in vitro) and during insulin induced hypoglycemia (in vivo)

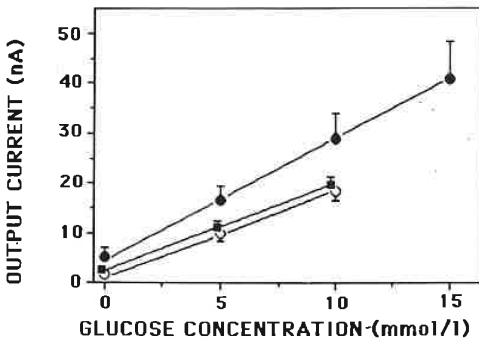


Fig. 4. Calibration curves determined in vitro before implantation (closed circles), and in vivo during insulin-induced hypoglycemia (open circles) or glucose-induced hyperglycemia (closed squares) $n = 7$; mean $\pm$ SEM

$r = 0.65$ for the sensitivity coefficients (Fig. 3C) and $r = 0.09$ for the background currents (Fig. 3D), *in vitro* before implantation vs. *in vivo* calibration 1. As a consequence, the calibration curves obtained *in vivo* and *in vitro* were considerably different (Fig. 4).

Determination of subcutaneous glucose concentration

Figure 5 shows the blood glucose concentrations and estimations of the subcutaneous glucose concentrations, calculated from the sensor signal, during the experiments of insulin injection and glucose infusion. Subcutaneous glucose reflected blood glucose, with a 5 min time lag, when calculated by taking into account the *in vivo* values of the sensitivity co-

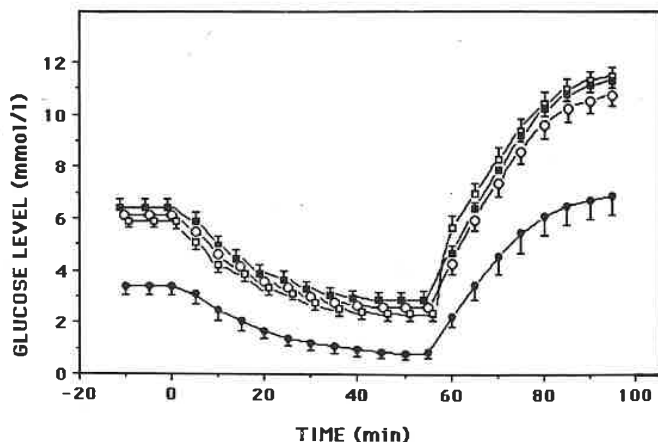


Fig. 5. Apparent subcutaneous glucose concentration, following insulin injection (time 0 min) and during glucose infusion started at 55 min. Subcutaneous levels were calculated with the calibration parameters determined *in vivo*, during insulin induced hypoglycemia (open circles) or glucose infusion (closed squares), and *in vitro* before implantation (closed circles). Concomitant blood glucose concentration is represented as open squares. ($n = 7$, mean $\pm$ SEM)

efficient ($\Delta I/\Delta C$) and background current (I_0), determined either with the pre- and post-insulin injection plateaus or the pre- and post-glucose infusion ones. By contrast, when calculated on the basis of the *in vitro* values of these parameters, determined just before implantation, the subcutaneous glucose concentration was much lower throughout the experiment than was the concomitant blood glucose: 3.4 ± 0.4 mmol/l before insulin injection ($n = 7$; $p < 0.001$ as compared to blood glucose), 0.8 ± 0.2 mmol/l after insulin injection at 50 min ($p < 0.01$), and 6.9 ± 0.8 mmol/l after glucose infusion at 95 min ($p < 0.001$).

Discussion

Until recently, the calibration of a subcutaneously implanted glucose sensor was not easy to perform because the relationship between the subcutaneous glucose concentration, which is detected in the form of an electric current, and the concomitant blood glucose concentration remained obscure. We have previously shown in the rat that the variations of this

"apparent subcutaneous glucose concentration" detected by the sensor closely follow those of blood glucose with a 5-min time lag, and that under stable situations both values are almost identical [1, 2]. Recently, evidence has been accumulated that under stationary conditions the true subcutaneous glucose concentration is indeed identical to the concomitant blood glucose level: investigations performed by means of subcutaneous implants such as cotton threads [3] and capillary filtrate collectors in dogs [4], and microdialysis catheters in humans [5], were able to show a good correlation between subcutaneous and blood glucose concentrations, with a 5 to 10 min delay for equilibrium. A practical consequence of these findings is that blood glucose values, determined under stationary conditions (a 5 to 10 min plateau), can be used to calibrate a subcutaneously implanted glucose sensor.

The aim of this work was, firstly, to compare retrospectively the apparent subcutaneous glucose concentrations calculated with *in vitro* or *in vivo* calibration parameters, secondly, to compare the apparent subcutaneous glucose concentration calculated with *in vivo* calibration parameters obtained either in hypoglycemic or hyperglycemic conditions. The *in vitro* calibration curve determined before implantation was found to be very different from the *in vivo* calibration curve calculated during insulin induced hypoglycemia (Fig. 4). This difference in calibration curves was due to differences both in sensitivity coefficients ($\Delta I / \Delta C$) and in background currents (I_0) as can be seen on Tab. 1. The *in vitro* calibration parameters, determined in phosphate buffer, were not correlated with those obtained *in vivo*, and the sensitivity coefficient was higher *in vitro* than *in vivo*. These data confirm those previously obtained under the same experimental conditions [2], but contrast with the data obtained with sensors calibrated *in vitro* in physiological saline. In the latter experiments the *in vitro* and *in vivo* background currents were correlated, and the *in vivo* sensitivity coefficients were higher than the *in vitro* ones [1]. Indeed the choice of a buffer can modify the *in vitro* characteristics of a given sensor (data not shown), this being an additional reason for calibrating an implanted sensor with *in vivo*-determined calibration parameters. When an apparent subcutaneous glucose concentration was calculated from the sensor signal by taking into account the *in vitro* calibration parameters, it remained throughout the experiments much lower than the concomitant blood glucose ($p < 0.01$ at any moment, Fig. 5). The use of *in vitro* calibration parameters might be one explanation for the low values of subcutaneous glucose concentration, as compared to concomitant blood glucose, observed by SHICHIRI et al. in dogs [6] and in diabetic patients [7] and by CLAREMONT et al. in pigs [8].

When the subcutaneous glucose concentration was calculated by using the *in vivo* parameters, determined either during insulin induced hypoglycemia or glucose infusion, it remained very close to the true blood glucose concentration with a 5-min time lag (Fig. 5). This may have practical consequences for the *in situ* calibration of a subcutaneously implanted sensor in clinical practice: the two point *in vivo* calibration could be performed, by measuring blood glucose and comparing it to the sensor output current, before and after meals or the ingestion of slow absorption index foods.

In conclusion, of the three possible ways of calibrating a subcutaneously implanted glucose sensor, namely *in vitro* calibration, one-point *in vitro*/one-point *in vivo* calibration and all *in vivo* calibration, only the latter makes it possible to avoid the bias introduced by differences in sensor characteristics observed *in vivo* versus *in vitro* conditions. This all *in vivo* calibration, performed both in hypoglycemia and hyperglycemia levels, allowed for the calculation of the apparent subcutaneous glucose concentration which approached very closely, in both cases, the true blood glucose concentration. This calibration procedure could easily be performed in routine clinical use of the sensor in diabetic patients.

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