Originals

Determination of peritoneal glucose kinetics in rats: implications for the peritoneal implantation of closed-loop insulin delivery systems

G. Velho, Ph. Froguel and G. Reach

Service de Diabétologie, Hôtel-Dieu, Paris, France

Summary. Peritoneal glucose kinetics were evaluated in the anaesthetized rat, to assess whether the peritoneal cavity would be a suitable site for the implantation of membrane-protected islets of Langerhans (bioartificial pancreas) or the glucose sensor of an artificial B cell. Glucose was measured in peritoneal fluid samples aspirated by needle puncture. Basal peritoneal and blood glucose concentrations were identical in 16 h fasted ($n=4$) and non fasted ($n=3$) animals. After 10 min of an i.v. glucose infusion ($n=15$) the increment in peritoneal glucose concentration was $63 \pm 3\%$ of the increment in blood glucose concentration and both values were significantly correlated ($r=0.92$; $p<0.001$). After 10 min of glucose clamping (12.6 ± 0.8 mmol/l), the increment in peritoneal glucose concentration was $69 \pm 3\%$ ($n=5$; $p<0.05$) of the increment in blood glucose concentration. In three additional experiments it was $93 \pm 3\%$ of the increment in blood

glucose concentration (NS), after 30 min of glucose clamping (8.0 ± 0.5 mmol/l). Peritoneal glucose concentration monitored by a glucose sensor: (a) followed blood glucose sluggishly during a glucose clamp ($n=5$), confirming the data shown above, (b) followed blood glucose with a 5 min delay and reached the same plateau after the intravenous injection of 1U insulin ($n=3$; NS). We conclude that peritoneal glucose reflects blood glucose at basal state and during variations of glycaemia, nevertheless, presenting heterogeneous kinetics. These kinetics might be appropriate for a bioartificial pancreas but not for an in vivo calibration procedure, of a peritoneally implanted glucose sensor.

Key words: Peritoneal, rat, glucose kinetics, closed-loop insulin therapy, glucose sensor, bioartificial pancreas.

The best site for implantation of the glucose sensor of a portable closed-loop insulin delivery system remains unsettled. The blood-stream which would allow for direct measurement of glycaemia, requires the maintenance of a patent arteriovenous shunt. Furthermore, poisoning of the sensor surface by direct blood contact, resulting in diminished sensor sensitivity and increased response time, can be expected within hours of implantation [1]. Extravascular fluid provides a much more suitable environment for glucose sensing than the blood-stream, being almost void of the clotting elements responsible for sensor poisoning. In this way subcutaneous tissue can be a valid alternative for sensor implantation: it is easily accessible and, in steady conditions of glycaemia, the subcutaneous glucose concentration reflects blood glucose with a 5 min delay [2–4]. The peritoneal cavity has been proposed as a site for insulin administration, on the assumption that insulin absorption via the portal system would allow for the generation of a more physiological porto-peripheral gradient, and that it could present better kinetics of in-

sulin absorption than the subcutaneous tissue. Several studies of peritoneal insulin administration either by open loop [5–7] or closed-loop [8–10] insulin delivery systems support these assumptions.

Thus, the peritoneal cavity could be an alternative site for the long-term implantation of a glucose sensor if its kinetics of glucose transfer from the blood-stream were better than, or at least as good as, those of the subcutaneous tissue. Furthermore, the peritoneal cavity has also been proposed as a site for implantation of microencapsulated islets within an artificial membrane [11, 12], macroencapsulated islets within a capillary hollow fiber [13], as well as microencapsulated glycosylated insulin complexed to concanavalin A [14, 15]. Rapid kinetics of glucose transfer from blood to the peritoneal cavity would also be of fundamental importance for the appropriate closed-loop regulation of insulin secretion by these implants. Nevertheless, the relationship between peritoneal glucose concentration and blood glucose and its variations has never been detailed. Long-term (4–14 weeks) peritoneal implantation of dialysis

Fig. 1. Correlation between basal peritoneal and blood glucose concentrations in 16 h fasted ($n=4$, closed squares; Group A) and non fasted ($n=3$, open squares; Group B) animals. ($y=0.30+0.93x$; $r=0.99$; $n=7$)

bags in rabbits and baboons [16] and short-term peritoneal implantation of a glucose sensor in an anaesthetized rabbit [17] have suggested that the peritoneal glucose concentration was lower than blood glucose, but no statistically relevant body of data from kinetic studies are available.

Thus, the aim of this work was to investigate the variations in peritoneal glucose concentration during variations in blood glucose induced by i.v. insulin injection and glucose infusion. This study was performed in anaesthetized rats.

Material and methods

Animal preparation and experimental groups

Thirty-five male Wistar rats (250–300 g body weight, Janvier, Saint Berthevi, France) fasted over-night, were anaesthetized with pentobarbital (50 mg/kg i.p., Clin Midy, Saint Jean de la Ruelle, France), with polyethylene (Biotrol 3, Biotrol, France) and silicone (Silastic 602–135, Dow Corning, Midland, Mich, USA) catheters being inserted respectively into the left jugular vein and homolateral carotid artery for glucose or insulin injection, and for blood sampling for the determination of blood glucose concentration. Animals were kept

Table 1. In vitro and in vivo characteristics of the glucose sensor

In Vitro	
$\Delta I/\Delta C$ (nA/mmole $\cdot$ l $^{-1}$)	0.59 ± 0.08
I_0 (nA)	4.80 ± 0.92
Linearity (mmol/l)	20.0 ± 1.0
$t_{90\%}$ (s)	38 ± 2
In Vivo (peritoneal)	
$\Delta I/\Delta C$ (nA/mmole $\cdot$ l $^{-1}$)	0.62 ± 0.09
I_0 (nA)	2.49 ± 0.68

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 $t_{90\%}$. ($n=8$, mean $\pm$ SEM)

spontaneously breathing under a flow of carbogen (O₂:CO₂, 95:5) and warmed under a lamp throughout the experiments. Forty-five to sixty min after the operation (at least 30 min of blood glucose stability) one of the following procedures was performed:

1. The skin over the abdominal wall was incised and access to the peritoneal cavity was made through a hole made over the linea alba with a 23 Gauge needle; 40 μ l of peritoneal fluid was then drawn with a pipette (Eppendorf 20 μ l Hamburg, FRG), for glucose measurement ($n=4$ over-night fasted animals, Group A). This procedure was also performed in non-fasted animals ($n=3$, Group B).

2. A continuous i.v. glucose infusion, the rate of which was different on each experiment and ranged from 8 to 24 mg $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ (infusion pump Infu 362, Datex, Uhewiesen, Switzerland) was initiated. Ten min later, peritoneal fluid was drawn as described above ($n=15$, Group C).

3. Blood glucose was clamped at 12.6 ± 0.8 mmol/l (Coefficient of variation: 1.7 ± 0.5 ; $n=5$; mean $\pm$ SEM) by means of a variable-rate continuous i.v. glucose infusion (20 to 30 mg $\cdot$ kg $^{-1}\cdot$ min $^{-1}$), using a 2.5% glucose solution, and adjusting the flow rate according to the plasma glucose concentration determined every 5 min. Ten min after blood glucose had reached the plateau, peritoneal fluid was drawn as described (Group D). In three other animals, blood glucose was clamped at 8.0 ± 0.5 mmol/l (Coefficient of Variation: 2.7 ± 0.2 ; $n=3$; 12 to 15 mg $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ infusion rate) and peritoneal fluid was drawn 30 min after the establishment of the plateau (Group E).

4. The peritoneal cavity was accessed as described above and a needle-type glucose sensor was placed intraperitoneally. When the sensor's signal had stabilized, either a continuous i.v. glucose infusion (Group F; 20 to 30 mg $\cdot$ kg $^{-1}\cdot$ min $^{-1}$; $n=5$) or an i.v. insulin injection (Group G; 1 U, Endopancrine U40, Organon, Saint Denis, France; $n=3$) was performed. Ten minutes after blood glucose had reached a new plateau, the sensor was removed and peritoneal fluid was drawn as described.

Glucose assay in plasma and peritoneal samples

Throughout these experiments, blood samples (0.3 ml) were serially drawn through the carotid catheter, at 5 min intervals. Samples were immediately centrifuged and plasma glucose concentration determined with a glucose analyser (Beckman, Fullerton Calif, USA). The cell pellet was resuspended and reinjected into the animals. Glucose concentration in centrifuged peritoneal fluid samples was determined in the same way.

Needle type glucose sensor

The amperometric needle-type glucose sensor has been described elsewhere [4]. Briefly, it consisted of a silver/silver chloride coated steel needle (cathode) containing an isolated platinum wire (anode), the tip of which was covered with glucose-oxidase entrapped in cellulose acetate and reticulated with glutaraldehyde, and covered with a polyurethane layer. Its external diameter measured 1.2 mm. Before implantation 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, which was recorded by a two-channel pen-recorder (SE120, Goerz Electro, Vienna, Austria). The characteristics of the glucose sensors ($n=8$) are summarized in Table 1.

Sensor calibration

In the experiments using glucose sensors, a continuous profile of the peritoneal glucose level was calculated from the sensor signal by using an in vivo calibration method based on the comparison of two points

of peritoneal glucose concentration with concomitant points of sensor output. The first point of peritoneal glucose was extrapolated from the glycaemia at the beginning of the experiment, when stable for at least 30 min. Indeed, previous to these experiments with glucose sensors, we verified that under steady state conditions the peritoneal glucose reflects blood glucose (see below). The second point was obtained by directly measuring glucose in peritoneal fluid drawn at the end of the experiment. This calibration made it possible to calculate for each experiment, both an *in vivo* sensitivity coefficient, expressed in $\text{nA}/\text{mmol}\cdot\text{l}^{-1}$ glucose, and an *in vivo* peritoneal background current (Peritoneal I_0), i.e., the extrapolated signal corresponding to 0 mmol/l glucose (Table 1). The calculated peritoneal glucose concentration was obtained by subtracting from the sensor current, observed at a given time, the *in vivo* background current (Peritoneal I_0), and then by dividing the resultant current by the *in vivo* sensitivity coefficient.

Statistical analysis

All data in text and figures are given as mean $\pm$ SEM, and their statistical significance was assessed by the two-tail paired or unpaired *t*-test, when appropriate.

Results

Basal peritoneal glucose concentration

Figure 1 shows the correlation between peritoneal glucose concentration and blood glucose concentration, in fasting (Group A) and non-fasting (Group B) animals ($r=0.99$; $y=0.93x+0.30$; $n=7$). Fasting peritoneal glucose was 5.4 ± 0.3 mmol/l and blood glucose was 5.5 ± 0.3 mmol/l ($n=4$; NS) while the non-fasting values were, respectively, 7.7 ± 0.5 and 7.9 ± 0.5 mmol/l ($n=3$; NS).

Peritoneal glucose concentration during *i.v.* glucose infusion: punctual measurements

Figure 2 shows the correlation between the increments in blood glucose concentration (ΔBG) and those concomitant, in peritoneal glucose concentration (ΔPG), measured 10 min after the initiation of an *i.v.* glucose infusion, bringing blood glucose from its basal value of 5.5 ± 0.2 mmol/l to a 7.8 ± 0.3 mmol/l plateau at 30 min (Group C). ΔPG was calculated as the difference between the peritoneal glucose concentration reached at 10 min and the initial blood glucose concentration since, as is demonstrated in Figure 1, the latter value represents an estimation of the basal peritoneal concentration. Each experiment was performed with a different flow rate to yield different increments in blood glucose concentration. As can be seen, at 10 min, ΔPG was $63\pm3\%$ of concomitant ΔBG ($n=15$; $p<0.001$; $y=0.63x+0.03$; $r=0.92$).

Figure 3 shows blood and peritoneal glucose concentrations, during variable-rate continuous *i.v.* glucose infusions: (A) Blood glucose was raised from its basal

Fig. 2. Correlation between the increments in peritoneal glucose concentration (ΔPG) and in blood glucose concentration (ΔBG) 10 min after the initiation of an *i.v.* glucose infusion. Each experiment was performed with a different flow rate ($8\text{--}24$ $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $n=15$; Group C). $y=0.63x+0.03$; $r=0.92$

Fig. 3. Peritoneal Glucose (open circle) determined 10 min after blood glucose (closed circles) clamping at time 0 min (Coefficient of Variability: 1.7 ± 0.5 ; $n=5$; $20\text{--}30$ $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ infusion rate started at -30 or -25 min, Group D). Peritoneal glucose (open square) determined 30 min after blood glucose (closed squares) clamping at time 0 min (Coefficient of Variability: 2.7 ± 0.2 ; $n=3$; $12\text{--}15$ $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ infusion rate started at -20 or -15 min, Group E)

value of 5.6 ± 0.2 mmol/l and clamped at a 12.6 ± 0.8 mmol/l plateau ($n=5$, Group D). Ten min after blood glucose clamping, peritoneal glucose concentration was 10.5 ± 0.6 mmol/l , ΔPG being $69\pm3\%$ of ΔBG ($p<0.05$). (B) Blood glucose was raised from a 5.9 ± 0.6 mmol/l basal level and clamped at a 8.0 ± 0.5 mmol/l plateau ($n=3$, Group E). Thirty minutes after blood glucose clamping, the peritoneal glucose concentration was 7.7 ± 0.6 mmol/l , ΔPG being $93\pm3\%$ of ΔBG (NS).

Continuous monitoring of peritoneal glucose concentration by a glucose sensor

Figure 4 (upper panel) shows the continuous monitoring of peritoneal glucose concentration, during an *i.v.* glucose infusion which brought blood glucose from its basal level of 6.1 ± 0.2 mmol/l to a 9.6 ± 0.2 mmol/l

Fig. 4. Continuous monitoring by a glucose sensor of peritoneal glucose concentration (open squares) during variations of blood glucose concentration (closed squares) induced by an i.v. glucose infusion (upper panel; $20\text{--}30\text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $n=5$; Group F) or an i.v. injection of 1U insulin (lower panel; $n=3$; Group G)

plateau in 20 min ($n=5$, Group F). The peritoneal glucose concentration, measured by the glucose sensor, sluggishly followed blood glucose levels and was $8.3 \pm 0.2\text{ mmol/l}$ at 30 min ($p < 0.05$), i.e., 10 min after the establishment of the blood glucose plateau.

In additional experiments, following the i.v. injection of 1U insulin (Fig. 4, lower panel), the blood glucose concentration decreased from its basal level of $5.9 \pm 0.1\text{ mmol/l}$ to a 2.8 ± 0.3 plateau at 35 min ($n=3$, Group G). The peritoneal glucose concentration followed blood glucose, with a 5 min time lag, to reach a $3.0 \pm 0.3\text{ mmol/l}$ plateau at 35 min (NS). The ratio between the changes in the peritoneal glucose concentration (ΔPG) and those in blood glucose (ΔBG) after 10 min of a sustained blood glucose plateau, was significantly different at the end of the experiments of glucose infusion (Group F) and insulin injection (Group G): ΔPG was, respectively, $63 \pm 6\%$ ($n=5$) and $94 \pm 1\%$ ($n=3$) of concomitant ΔBG ($p < 0.05$).

Discussion

This work was undertaken to study the kinetics of peritoneal glucose concentration under varying blood glucose conditions induced by i.v. insulin injection and glucose infusion, with a view to evaluating the peritoneal cavity as a site of implantation for the sensing part of a closed-loop insulin delivery system. Rapid kinetics of the glucose signal at the site of glucose sensing are of paramount importance for an appropriate regulation of insulin delivery, whatever the nature of the closed-loop system, namely artificial (artificial B cell), chemical (concanavalin A-bound glycosylated insulin) or biological

(bioartificial pancreas). Sorensen et al. using a theoretical physiological pharmacokinetic model of glucose homeostasis controlled by a closed-loop insulin delivery system, showed that sensing blood glucose variations with a sensing delay of more than 15 min, would result in progressive loss of glucose regulation, exacerbation of hyperinsulinaemia, and increased insulin requirements [18].

Our results show that basal peritoneal glucose concentration reflects blood glucose (Fig. 1) in fasting as well as in non-fasting animals. These data contrast with those obtained by Wolfson Jr. et al. who implanted, for 4–14 weeks, cellulose dialysis bags (20 mm long, 6.4 mm internal diameter) in the peritoneal cavity of rabbits and baboons [16]. Glucose concentration in the fluid sampled from the bags every 3–10 days was found to be 44–74% of blood glucose. Nevertheless, due to the large volume of the bags, glucose concentration in the samples represented an integrated response over several days, with a possible dilutional effect, while blood glucose represented punctual measurements. Kessler et al. implanted a glucose sensor into the peritoneal cavity of anaesthetized rabbits, and observed that there was a linear relationship between glucose concentration in blood and peritoneal cavity. The peritoneal glucose concentration was 58% of that observed in blood [17]. However, an in vitro calibration of the sensor was used to estimate, from the sensor output current, the glucose concentration. The use of this procedure is questionable and might be an explanation for the low levels of glucose in the peritoneal cavity as compared to blood [4]. In the experiments with the glucose sensor shown above (Groups F and G) we used an in vivo procedure to calibrate the sensor, i.e., to calculate from the sensor output current the peritoneal glucose concentration. This calibration was based on the measurement, by an independent method (Beckman analyser), of two points of peritoneal glucose concentration. The first one was estimated from the glycaemia at the beginning of the experiment (see Fig. 1); the second one directly measured in the peritoneal fluid sample at the end of the experiment. Therefore, this calibration made it possible to determine the true glucose concentration inside the peritoneal cavity.

During i.v. glucose infusion experiments, we found that the variations in the peritoneal glucose concentration (ΔPG) were correlated to those in blood glucose (ΔBG), but presented slow kinetics. Indeed, after ten min of a glucose infusion which changed the glycaemia in the physiological range (0.5 to 2.5 mmol/l change, depending on the experiment) ΔPG was 63% of concomitant ΔBG (Fig. 2, Group C). Both values were well correlated over the range of glycaemia variation ($r=0.92$). Furthermore, again during glucose infusion, but 10 min after blood glucose had reached a new plateau, ΔPG was 69% of ΔBG (data from Fig. 3 upper curve, Group D). This sluggish response to blood glu-

glucose changes was best seen during the continuous monitoring of peritoneal glucose by a glucose sensor, performed in additional experiments (Fig. 4 upper panel, Group F): peritoneal glucose concentration started changing 5 min after the glucose infusion was initiated and ΔPG was 63% of ΔBG 10 min after blood glucose clamping. Thus, in group D, in which plasma glucose level was clamped at 12.6 mmol/l, and in group F, in which it was clamped at 9.6 mmol/l, the peritoneal glucose concentration was not fully equilibrated with plasma glucose concentration within 10 min of clamping. In contrast, when peritoneal glucose was measured 30 min after blood glucose clamping at 8.0 mmol/l, ΔPG was very close to ΔBG (93%; Fig. 3 lower curve, Group E; $p < 0.05$ as compared to Group F). These results might be due in part to the difference in the clamping levels. However, the comparison of groups D and F, in which the clamping levels were different and the ratio $\Delta PG/\Delta BG$ was not found to be significantly different at 10 min of blood glucose clamping, suggests that the length of clamping played a major role in these kinetics. Thus, at least at an 8 mmol/l clamping level, it can be estimated that 30 min represents the time lag required for the full equilibration of blood and peritoneal glucose concentrations. These results are consistent with the data in Figure 1, which shows that in both fasted and fed animals blood and peritoneal basal glucose concentrations were almost identical.

Contrasting results were obtained following an i.v. injection of insulin. Peritoneal glucose concentrations followed the variations in blood glucose with a time lapse of 5 min, as can be seen by continuous monitoring of peritoneal glucose with a glucose sensor (Fig. 4 lower panel). Indeed, the kinetics of peritoneal glucose following the insulin injection were faster than during glucose infusion, ΔPG reaching respectively, 94% and 63% of ΔBG after 10 min of a new blood glucose plateau.

There is no clear explanation for this difference in behaviour. It could not be related to any sensor calibration bias since, in both insulin injection and glucose infusion experiments, the glucose concentration was checked in peritoneal fluid samples at the end of the experiment. Furthermore, in control experiments we checked the drift in the response of peritoneally implanted sensors: under stable normoglycaemic conditions only a minimal drift was observed ($< 1\%/h$; data not shown). However, it is tempting to speculate that this difference in behaviour could be related to the very different physiological modifications induced by glucose infusion and insulin injection. While in the former situation the rise in the peritoneal glucose concentration depends on the glucose transfer from the circulation into a diffusion space, in the latter, the fall in peritoneal glucose concentration may also depend on the insulin-induced intracellular absorption of glucose by the peritoneal tissue itself.

In conclusion, peritoneal glucose concentration was shown to present heterogeneous kinetics during variations in blood glucose levels. If these data are confirmed in man, the implications for the peritoneal implantation of biological or chemical closed-loop insulin delivery systems may be different from those for the implantation of a glucose sensor or an artificial B cell. Currently, the available data on peritoneally implanted biological or chemical systems do not provide unquestionable evidence of the establishment of a closed-loop control of glucose homeostasis. Bioartificial pancreatic systems consisting of islets of Langerhans placed inside microcapsules [11, 12] or hollow fibers [13] have been implanted in diabetic mice and rats, and a chemical system (lectin-bound insulin complex, encapsulated in a dialysis bag) was implanted in diabetic dogs [15]. Blood glucose concentration was normalized in all these experiments, but there were no experimental data demonstrating an increase in plasma insulin in response to a glucose challenge. This normalization of blood glucose per se does not demonstrate that a closed-loop insulin delivery was established in these animals since polymeric insulin implants, which release insulin without any regulation, were also able to normalize blood glucose in diabetic rats [19, 20]. Of course, the experimental design of our study cannot provide evidence of the functioning of the entire closed-loop. Nevertheless, our data suggest that glucose sensing would be feasible in the peritoneal cavity: 60 to 70% of a change in blood glucose level is perceived within 10 min.

Finally, concerning the implantation of the glucose sensor of an artificial B cell, we face a different problem; all electrochemical sensing devices present drift and thus must be often calibrated. It would seem from our data that under these experimental conditions, the peritoneal cavity presents slower and more heterogeneous glucose kinetics than those of the subcutaneous tissue. These kinetics could make unfeasible an in vivo sensor calibration procedure based on the measurement of blood glucose. Indeed, it would be difficult to maintain blood glucose at a steady level, long enough to allow for the equilibration between peritoneal and blood glucose concentrations.

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Dr. G. Reach
Service de Diabétologie, Hôtel-Dieu
1 Place du Parvis Notre Dame
F-75004 Paris
France