A microdialysis method allowing characterization of intercellular water space in humans

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LÖNNROTH, P.-A. JANSSON, AND U. SMITH. A microdialysis method allowing characterization of intercellular water space in humans. Am. J. Physiol. 253 (Endocrinol. Metab. 16): E228-E231, 1987.—To evaluate the usefulness of the tissue-microdialysis technique in humans, the glucose concentration in the intercellular water space was measured in the abdominal subcutaneous region in healthy subjects. A 30 x 0.3 mm dialysis fiber with a 3,000 MW cutoff was used. The dialysis catheter was calibrated in vivo by perfusing it with isotonic saline and four to five different glucose concentrations (0-5 mM). The perfusate was collected in 6-min fractions. Regression analysis of the results of the calibration yielded the perfusate glucose concentration, which was in equilibrium with the surrounding tissue. Validation experiments showed that this value could be precisely measured and represented the intercellular glucose concentration. The recovery of glucose in the dialysate (dialysate glucose concentration/medium) during the calibrations in vivo was only approximately one-half of that in vitro (recovery factors 0.28 vs. 0.44, respectively). Under steady-state conditions, the intercellular glucose concentration was similar to the glucose levels in the cubital vein. It is concluded that this microdialysis technique is useful tool allowing measurements of metabolically active substances in the intercellular water space in vivo provided that the calibrations are properly performed.

tissue microdialysis; adipose tissue; glucose

CLINICAL EXPERIMENTAL RESEARCH has by necessity been restricted to performing measurements on samples drawn from the blood rather than from the more relevant intercellular space. Concentrations in the blood of different substances may not reflect the concentrations at the cellular level depending, for instance, on degree of protein binding, capillary permeability, and kinetic differences in the various distribution volumes. Until recently, measurements in the intercellular water space in vivo have been limited to compounds that can be detected by electrodes and/or biodetector enzyme systems. A major opening in this field were the recent findings that in situ microdialysis of the rat brain could be used to measure metabolic events at the cellular level (1, 3). The technique is atraumatic to the tissue and permits stable dialysis conditions for several days (1, 3). However, to calculate the tissue concentrations of the different compounds measured it has been assumed that the recovery in the perfusate of a certain concentration gradient across the dialysis membrane in vivo is equal to that obtained in the calibration experiments performed in vitro (1, 3).

In the present study we have used for the first time the in vivo tissue-dialysis technique in human subjects. A simple method to characterize the true recovery of any compound measured by the microdialysis technique in vivo has been developed.

METHODS

Subjects. Ten healthy male students of normal weight, age 23-34 yr, participated in the study and were investigated at one or two occasions. All subjects gave their informed consent and the study was approved by the Ethical Committee of the University of Gothenburg.

Subcutaneous tissue dialysis. A single dialysis tubing (30 x 0.3 mm, Cuprophan B4AH, 3,000 MW cutoff, Cobe, Denver, CO) was glued to a nylon tubing with cyanoacrylate and sterilized. The outlet of the nylon tubing had a standardized length of 50 mm (Fig. 1A). No anesthesia of the skin was required. The inlet of the nylon tubing was connected to a precision pump (SAGE Instruments, Boston, MA) and perfused with isotonic saline at a rate of 2.5 µl/min. After an equilibration period of 30 min, the dialysate was collected in 6-min fractions. Venous blood samples were drawn from a polyethylene catheter placed in the cubital vein.

Glucose analysis. The glucose concentration in plasma and dialysate was measured with the glucose oxidase method (Kabi, Stockholm, Sweden). Deproteinized plasma or dialysate was added and the absorbance measured in a spectrophotometer at 450 nm.

Characterization of dialysis probe in vitro. To characterize the dialysis probe, the catheter was placed in a saline solution with different concentrations of glucose. As shown in Fig. 2 the percentage recovery of glucose in the dialysate in vitro was constant (44 ± 2%, mean ± SE) over the concentration range tested. The sensitivity of the dialysis probe to record rapid changes of the glucose concentration was studied by switching the catheter from 5 to 10 mM glucose. When 2-min fractions were collected 16% of the change in the glucose concentration was recorded in the perfusate after 2 min and 97% after 4 min (Fig. 2B). Thus 72% of an immediate change of the glucose concentration in the surrounding medium will be recorded in a 6-min fraction (Fig. 2C), whereas 100% of an ongoing change will be detected after
8 min at the latest.

Estimation of intercellular glucose concentration. The glucose concentration in the dialysate mirrors the recovery of the glucose gradient by the dialysis membrane. Because the recovery of glucose in vitro was constant over a glucose concentration range of 0.25–5 mM, provided that the flow rate of the dialysis fluid is constant, a linear relationship is established between the glucose concentration in the perfusate and the concentration gradient over the dialysis membrane. If, then, the dialysis probe is perfused with different concentrations of glucose, a linear relationship is established between the net increase of the glucose concentration in the perfusate and the concentration of glucose in the inlet of the tubing (Fig. 3). By using regression analysis the concentration of glucose in the probe not resulting in any net influx of glucose in the perfusate can be calculated. At this glucose concentration the gradient across the dialysing membrane is zero, and thus the glucose concentration is in equilibrium with that of the surrounding tissue. The mean recovery of glucose is obtained from the slope of the line (Fig. 3).

The linear regression analyses were based on four to five different concentrations using the least squares method. The extracellular glucose concentration was calculated when the correlation coefficient was 0.9 or greater. This was the case in at least 90% of the experiments.

RESULTS

Intercellular glucose concentration under steady-state conditions. Figure 4 depicts results from four experiments where the subjects were investigated with two dialysis probes placed on each side of the umbilicus. Each probe was perfused with four to five different glucose concentrations. The equilibrium glucose concentration was in each subject similar to the glucose concentration in the cubital vein. The mean difference of the glucose concentration in the tissue and blood was $6 \pm 1\%$ (mean $\pm$ SE). If the result from each dialysis probe was counted as a single experiment, the relative difference was $12 \pm 2\%$ ($n = 8$). The mean recovery of the glucose gradient across the dialysis membrane was $22 \pm 2\%$.

As shown in Fig. 4, the points when the dialysis probe was perfused with saline without glucose were below the regression line in all four subjects. In this situation the recovery of glucose is lower than the mean ($20 \pm 1\%, n = 8$). When the linear regression analyses were performed without these measure points, the mean recovery increased to $28 \pm 2\%$. In addition, the accuracy of the calculated tissue glucose concentration was improved because the mean difference between the blood and tissue glucose concentrations was only $8 \pm 3\%$ ($n = 8$) when the measurements were performed with glucose in the perfusate.

Table 1 shows data from experiments where only one or two different glucose concentrations were added to the perfusate and the tissue glucose concentration cal-

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**FIG. 1.** Schematic picture of the dialysis probe. A $30 \times 0.3$ mm dialysis membrane was attached with acrylate to a nylon tubing and sterilized (A). Dialysis probe was placed in subcutaneous tissue with a small injection needle and connected to a precision micropump (B).

**FIG. 2.** Characterization of the dialysis probe in vitro. A: dialysing tubing was placed in a vessel containing isotonic saline with different glucose concentrations and perfused with a flow rate of $2.5 \mu l/min$. Data are means $\pm$ SE of 4 consecutive experiments. $y = 0.447x - 0.042; r = 0.997$. B: glucose concentration in dialysate after a rapid change in the ambient medium. Figures are expressed as percentage change in consecutive 2-min fractions. Data are means $\pm$ SE of 3 experiments. C: same experiment as in B but glucose concentrations were measured in consecutive 6-min fractions.
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O a1 a2 aY

Glucose concentration in perfusate

FIG. 3. Mathematical model for estimating dialysis recovery. When concentration gradient over dialysis membrane is altered by a change in glucose level in perfusate (a) a corresponding change in net increase of the concentration in the dialysate (open triangle) is recorded. By varying the concentration gradient repeatedly and by recording the net influx of the compound at every point a linear relationship is established. Intercept on x-axis (a,) indicates glucose concentration in perfusate at equilibrium with surrounding medium (= tissue concentration). Slope of line (A_/a,) gives the dialysis recovery of the compound.

FIG. 4. In vivo dialysis of glucose from abdominal subcutaneous tissue in 4 subjects (A–D). Figures are means ± SE of 2 dialysis probes placed on each side of umbilicus. Blood/tissue glucose concentrations (mM) were 4.3/4.1 (A), 3.7/3.4 (B), 3.9/4.2 (C), and 4.4/4.6 (D).

Glucose concentration in perfusate (mM)

VALUES ARE MEANS ± SE. Effect of different concentration gradients during the tissue dialysis with a single dialysing probe is shown. A mean recovery of 0.20 or 0.28 was used for calculating the tissue glucose concentrations when the dialysis probe was perfused with 0 and 3 mM glucose, respectively.

To adequately measure the intercellular concentrations it is essential that the dialysis probe is properly calibrated in vivo. Recovery of glucose was much smaller when the calibration experiments were performed in vivo than in vitro. It was also found that the recovery was a function of the concentration gradient across the dialysis membrane. These characteristics of the dialysis probe have previously not been taken into account (1, 3) but must be adequately dealt with to calculate the intercellular concentrations properly. The present study shows that the problems are easily handled by changing the concentration equilibrating in the perfusate before dialysis.

The mechanisms for the change in recovery as a function of the concentration gradient across the dialysis membrane are unclear. It could be speculated that the net inflow of glucose in this situation is sufficiently large to drain the intercellular water space of glucose. This, in turn, would give rise to local concentration gradients in the tissue. However, a good estimate of the tissue glucose concentration was possible under steady-state conditions when the tissue was dialysed with saline alone, without any addition of glucose. Further support for the maintenance of steady-state conditions also in the presence of a high concentration gradient across the dialysis membrane was the observation that the glucose recovery was highly reproducible (20 ± 1%, n = 8). Furthermore, control experiments have shown that the recovery of glucose remains constant for at least 1 h in the presence of a large concentration gradient across the dialysing membrane (data not shown). Thus tissue depletion of glucose does not appear to occur under steady-state conditions. When microdialysis was performed with a small concentration gradient (1–4 mM glucose added to the perfusate) steady-state conditions were also maintained. This was shown in several experiments performed for 3 to 4 h by the linear relationship between net influx of glucose and the concentration gradient.

The glucose concentration in the subcutaneous tissue was equal to that in venous blood under steady-state conditions when the probe was perfused with 0 or 3 mM glucose, respectively.

TABLE 1. Blood and tissue glucose concentrations under steady-state conditions

<table>
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<th>Number of Concentration Gradients</th>
<th>Perfusion Glucose Concentration, mM</th>
<th>Estimated Tissue Glucose Concentration, mM</th>
<th>Venous Blood Glucose Concentration, mM</th>
<th>Mean Difference Between Tissue and Blood, %</th>
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<td>3.9±0.3</td>
<td>4.1±0.2</td>
<td>12±2</td>
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</table>

DISCUSSION

The present study demonstrates for the first time the usefulness of the microdialysis technique for characterizing the intercellular water spaces in humans in vivo.
conditions. Similar data were recently reported from measurements in the subcutaneous tissue with a glucose oxidase electrode (2).

In summary, the microdialysis method appears to be a most useful tool for measuring the intercellular concentrations of different substances in humans. Provided that the true recovery of every compound is adequately measured as described in this paper the method offers a unique approach to study ongoing metabolic events at the cellular level in vivo.

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REFERENCES

