Reverse Iontophoresis: Development of a Noninvasive Approach for Glucose Monitoring

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Solvent flow generated during iontophoresis can be used to convect neutral molecules through the skin, thereby greatly enhancing their flux. This concept was exploited to realize noninvasive glucose measurement by its iontophoretic extraction from the subcutaneous tissue. The hypothesis was tested *in vitro* using hairless mouse skin. The dermal surface was bathed with a glucose solution; chambers on the epidermal surface housed the current delivery electrodes. Iontophoresis (at 0.36 mA/cm^2) was performed for 2 hr, at the end of which the solutions in contact with the electrodes were analyzed. The amount extracted was proportional to the glucose solution concentration bathing the dermis. Higher radioactivity levels were found at the anode than at the cathode, possibly because of glucose metabolism during its outward transport across the skin. Glucose biotransformation results in negatively charged metabolites which migrate to the anode. Two sensitive glucose sensors were developed; one was selective for glucose, the other for glucose and related compounds. Both sensors indicated the presence of glucose at the cathode but an abnormally high value was also recorded at the anode. This signal, however, was not due to glucose but rather to electroactive ascorbate withdrawn from the skin. Finally, a system has been developed with which glucose can be extracted noninvasively from the subcutaneous tissue and unambiguously measured. Whether iontophoretic glucose sampling *in vivo* will be equally successful remains to be answered.

KEY WORDS: transdermal; iontophoresis; noninvasive; blood glucose; monitoring; biosensor.

INTRODUCTION

Blood glucose level monitoring is a crucial daily aspect of a diabetic's life. Present procedures for obtaining such information, however, are invasive and painful. Development of a noninvasive approach, requiring little or no patient participation, would represent a significant advance. In an earlier report (1), we presented preliminary information on the possibility of glucose monitoring via transdermal, "reverse" iontophoresis. Here we expand upon these findings and demonstrate that available biosensing technology brings us closer to the realization of the long-term objective.

Although glucose is present in the body at reasonable levels, the very low passive permeability of this highly polar and water-soluble species across the lipophilic skin barrier precludes the possibility of surface collection as a viable means to acquire real-time information about circulatory systemic levels. However, it is now established that application of a potential difference across the skin (i.e., passage of a small current through the membrane) enhances the transport of both charged and neutral molecules (2–5). This iontophoretic enhancement of uncharged species results from current-induced solvent flow: as the electrostatically driven ions move through the medium, there is momentum transfer to adjacent solvent molecules, inducing flow in the latter. Neutral solute transport is thus enhanced by the convective action of the solvent. In an uncharged membrane the concentrations of anions and cations are equal, and solvent flow in one direction associated with positively charged species is exactly canceled by flow in the opposite sense associated with ions of negative charge; hence, no net flow occurs. But the skin, under physiological conditions, supports an overall negative charge and is permselective, therefore, to cations. Consequently, in iontophoresis, solvent flow moves preferentially from anode to cathode (6–8); hence, one would logically anticipate the feasibility of reverse iontophoretic glucose extraction and monitoring to be demonstrable most effectively at the cathode.

In this paper, we examine the preceding hypothesis in an *in vitro* system (1,9). It follows that the relevance of our results depends upon there existing a correlation between cutaneous glucose concentrations and systemic levels, a relationship which has been confirmed very recently (10–13).

MATERIALS AND METHODS

Diffusion Cell

The *in vitro* system employed has been designed to mimic the *in vivo* situation (1,9). Thus, the electrode chambers were situated on the same, epidermal side of a single piece of skin, while the dermal surface of the tissue was bathed with a physiological buffer containing glucose at the desired level. Current was delivered via electrodes positioned into the electrode chambers and immersed in appropriate electrolyte.

Electrodes

The iontophoretic current was supplied by the reversible Ag/AgCl couple. Because the Ag/AgCl electrode is nonpolarizable, a large current can be delivered with minimum energy input. The power supply employed will not allow the anodic and cathodic background limits of Ag/AgCl electrodes to be approached, and pH changes in the supporting electrolyte, associated with electrolysis of water, do not occur.

The electrodes were prepared by dipping silver wire (1-mm diameter, 99.99% pure; Aldrich Chemical Company, Milwaukee, WI), which had been cleaned with emery paper, into molten AgCl (99%; Aldrich Chemical Co.). To ensure that silver depletion from the anode during iontophoresis did not cause stability problems, a large reservoir of silver was generated electrochemically *in situ* by cathodizing the electrode overnight against a platinum anode.

Electrolyte

The background electrolyte in the electrode chambers

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and bathing the dermal side of the skin was 25 m*M*, pH 7.4, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer containing 133 m*M* NaCl (Sigma Chemical Co., St. Louis, MO). Glucose or, in some cases, ethanol was added to the dermally perfusing medium at the desired concentration. The glucose analyte was either completely "cold" or spiked with 0.4 μCi/mL of ^{14}C-uniformly labeled chemical (ICN Biomedical Inc., Irvine, CA). Radiochemical purity of ^{14}C was checked by TLC and was >99.9% pure. In the ethanol experiments, a small amount of radiotracer (^{14}C-ethanol) was incorporated into the ethanol solution in electrolyte, which was used to bathe the dermal skin surface. To confirm the direction of electroosmotic solvent flow during the iontophoretic experiments, limited studies examined the movement of tritiated water under the influence of the applied current.

Skin Source

Full-thickness skin, excised and used immediately after sacrifice, from female hairless mice (HRS/hrs, Simonsen Laboratories, Gilroy, CA) was used in all experiments.

Current Delivery

All experiments employed a constant current of 0.4 mA (0.36 mA/cm^2). Current was delivered from a custom-built power supply interfaced to a Macintosh IICi via LabView (National Instruments, TX) software.

Procedures

The diffusion cell was assembled, with the dermal bathing solution containing the appropriate analyte concentration, the electrodes were inserted into the electrolyte in the electrode chambers, and current was passed for 2 hr. At the end of this time, the electrode solutions were carefully removed for analysis of analyte.

Analytical

Initial experiments with glucose, and all those with ethanol, utilized ^{14}C-labeled solutes. Quantitation of analyte extracted by reverse iontophoresis employed liquid scintillation counting. Thus at the end of the sampling period the electrolyte in the electrode chambers was mixed with an appropriate volume of scintillation cocktail (Beckman, Fullerton, CA) and radioactivity was determined in a liquid scintillation counter.

Because the results from the glucose sampling measurements using ^{14}C radiolabel were unexpected (see below), experiments were repeated with cold analyte using electrochemical detection methodology. Two glucose biosensor approaches were considered.

Platinum/Glucose Oxidase Electrode. Glucose reacts very specifically with the enzyme in the presence of oxygen to produce gluconic acid and hydrogen peroxide. H_2O_2 is then easily oxidized to water and O_2 on a platinum electrode, resulting in a current that is directly proportional to the original glucose concentration. The response of this system to glucose was highly specific and sensitive, as shown by the calibration curve in Fig. 1, which indicated linearity into the submicromolar glucose concentration range. Interference in the operation of this sensor results from the presence of species which are directly electroactive on a platinum surface at a potential of +0.7 V (vs Ag/AgCl).

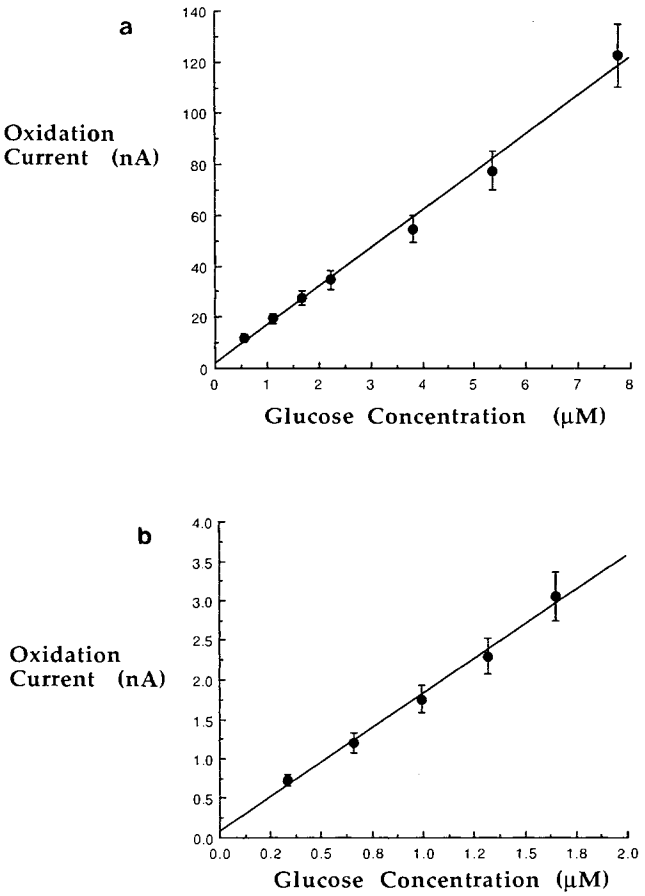

Fig. 1. Calibration curves for (a) the platinum-glucose oxidase and (b) the modified copper electrode biosensors. The results shown represent the mean ± SD of four replicate measurements.

Modified Copper Electrode. On a modified copper surface, glucose can be oxidized at high pH and the system can be used to form the basis of a very sensitive sensor (see calibration curve in Fig. 1). The high pH enhances the formation of an unstable copper species and also favors the binding of molecules with hydroxyl groups. A redox reaction between the two bound moieties produces an oxidation current, which is proportional to the concentration of the molecule containing the hydroxyl groups. It should be noted that, although sensitive, the modified copper electrode is not specific to glucose; rather it is able to oxidize many organic species with multiple hydroxyl groups.

RESULTS AND DISCUSSION

Using radiolabel detection, the passive transport of glucose from a 5 m*M* solution on the dermal side of the skin to the epidermal surface was negligible. Current passage for 2 hr, on the other hand, produced, as expected, appreciable ^{14}C extraction to the cathode (Fig. 2). However, unexpectedly, the detected radioactivity levels in the anode chamber were higher (Fig. 2) than those at the cathode. Electroosmotic flow under identical conditions, though, was confirmed to be in the predicted anode-to-cathode direction; the

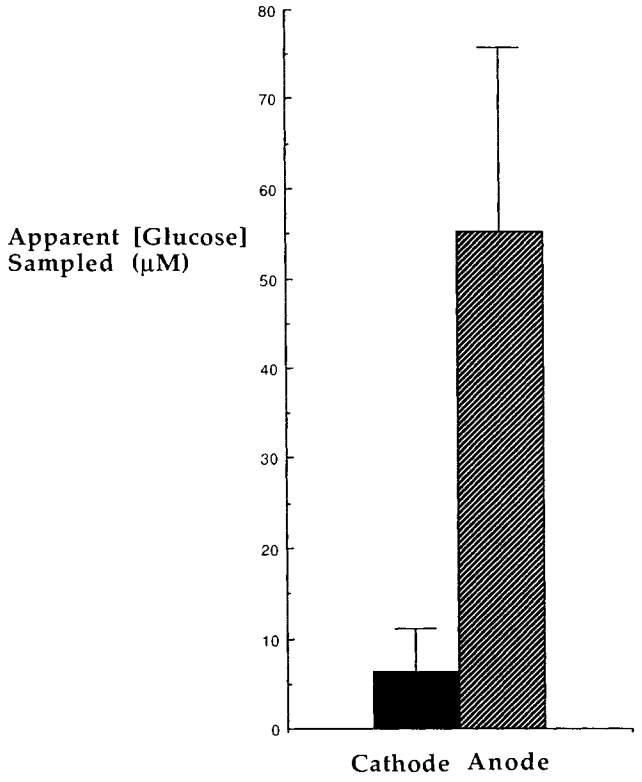

Fig. 2. Apparent extraction of glucose by reverse iontophoresis in 2 hr. The concentrations on the *y* axis are calculated assuming that all radioactivity measured in the electrode chambers is glucose. The results shown represent the mean ± SD of 10 replicate measurements.

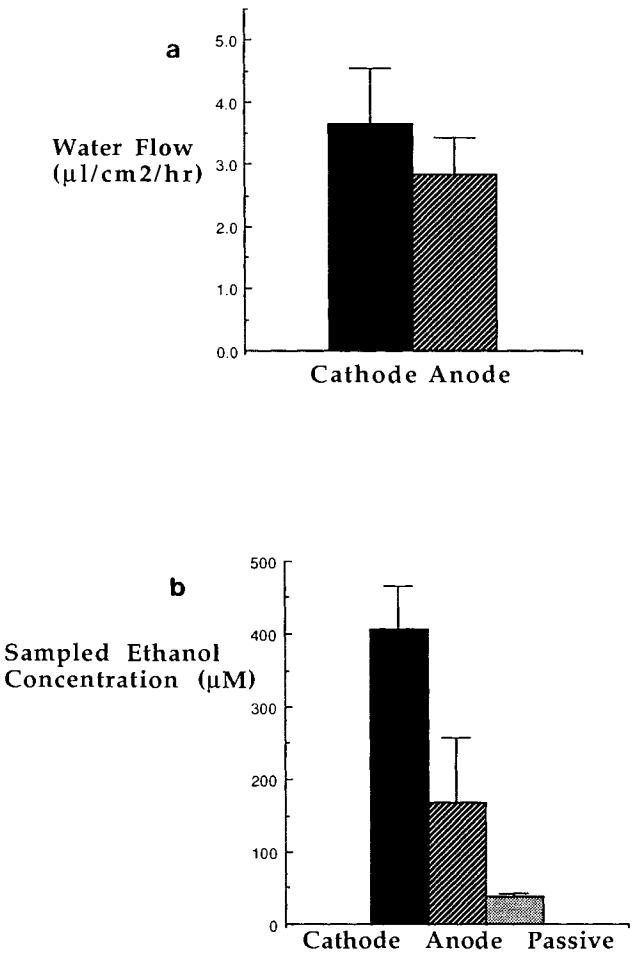

Fig. 3. Reverse iontophoretic extraction of (a) tritiated water and (b) ^{14}C-labeled ethanol in 2 hr. The results shown represent the mean ± SD (n = 8 for water; n = 6 for ethanol).

electrotransport of both tritiated water and ^{14}C-labeled ethanol showed preferential "extraction" to the cathode (Fig. 3). The concentration of ethanol perfused subdermally in this experiment was 18 m*M*, which is equivalent to the legal driving limit in California. The passive permeability coefficient of ethanol in our control experiments here was deduced to be approximately 2.7×10^{-3} cm/hr, a value that compares reasonably with that in the literature [approximately 4.0×10^{-3} cm/hr (14)].

A logical explanation for the high levels of radioactivity found at the anode was that negatively charged metabolites of glucose (such as lactate and pyruvate) were being formed by interaction of the analyte with the skin and were then being drawn (in the iontophoretic fashion) toward the positive electrode chamber. Glucose metabolizing enzymes are present in the epidermis (15–17) and the formation of lactate over water and carbon dioxide is favored (15). Limited, preliminary high-performance liquid chromatography experiments lend support to this suggestion (data not shown).

More encouraging, however, was the reproducibility and linearity of the ^{14}C-extraction process by reverse iontophoresis (Fig. 4). Providing further evidence for the metabolism argument presented above was the observation that, at the highest (and admittedly, unphysiological) subdermal glucose levels used, the total radioactivity extracted at the cathode exceeded that at the anode, implying perhaps that saturation of the metabolic processes in the skin had been reached.

The kinetics of the extraction process were also examined in these radioactivity experiments: the electrode chambers were, in this case, assayed every 30 min for extracted material. The results (Fig. 5) showed that, even within a half-hour, appreciable electrotransport could occur. Not surprisingly, given that electrostatic attraction (as opposed to electroosmosis) was apparently driving transport to the anode, the kinetics of ^{14}C appearance in the positive electrode chamber was swifter, with essentially no demonstrable lag time.

In order to obtain specific measurements of glucose extraction be obtained, reverse iontophoresis experiments were next performed in which the dermal bathing solution was 5 m*M* glucose without radiolabel tag; analysis of the electrode chambers after 2 hr of current passage utilized the two glucose-sensitive electrochemical approaches described under Materials and Methods. The results are summarized in Table I; once again, confounding observations were obtained. For both sensors, greater apparent glucose levels were found at the anode. In the case of the modified copper electrode, its lack of specifity probably accounted for the data observed. Glucose metabolites are also active (to varying degrees) on this electrode, and the current registered, therefore, represents a combined signal from several spe-

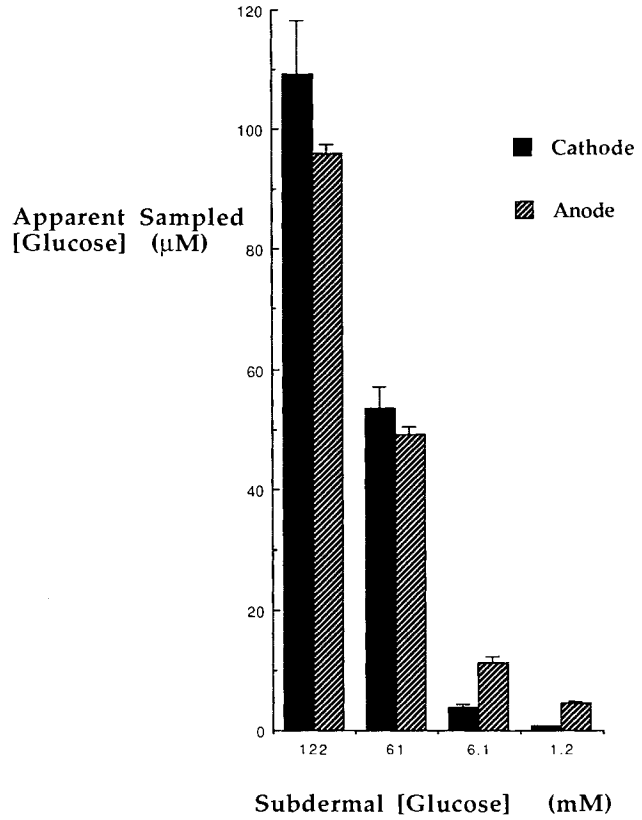

Fig. 4. Apparent concentration dependence of reverse iontophoretic extraction of ^{14}C-labeled species at anode and cathode chambers following subdermal perfusion of the skin with a wide range of ^{14}C-glucose concentrations. The results shown represent the mean ± SD of two replicate measurements.

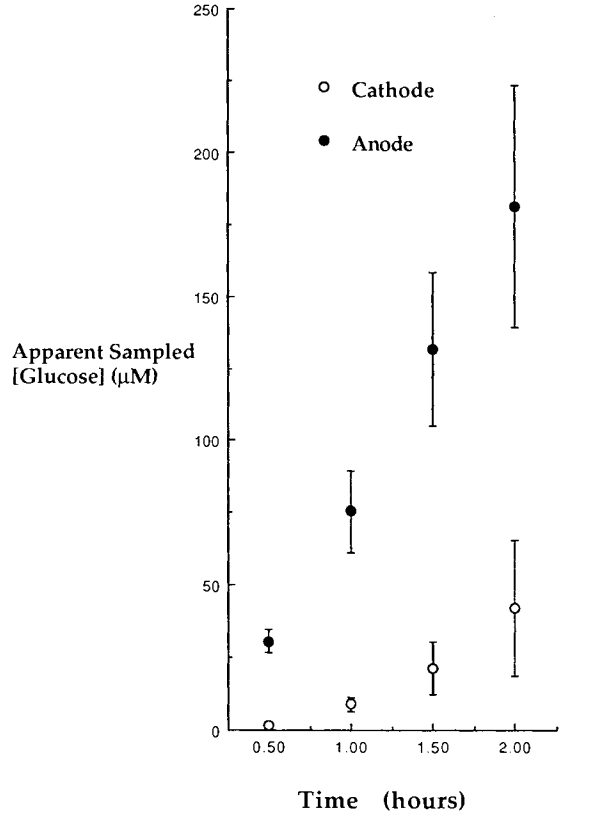

Fig. 5. Kinetics of apparent reverse iontophoretic extraction of glucose to anode and cathode chambers with the dermal skin surface bathed in 5 m*M* glucose solution. The results shown represent the mean ± SD of six replicate measurements.

cies. The high signal at the anode is consistent with the radioactivity data reported above. Attention was focused, as a result, on the platinum–glucose oxidase (Pt-GOD) sensor. A control reverse iontophoresis experiment was performed in which no glucose was added to the dermal bathing solution. While the cathodal current was reduced (but still nonzero), the anodal current remained high. Thus, the source of the signal from the anodal chamber was not a glucose-related species. Review of the electrochemical literature indicated the electroreactivity of a number of biologically present species on a bare platinum electrode at the potential used for glucose analysis by the Pt-GOD sensor. One prime candidate was ascorbic acid, which is readily oxidized to the dihydroquinone (18–20). Subsequently, therefore, the reverse iontophoresis experiment with 5 m*M* glucose was repeated. At the end of current passage, the solutions in the electrode chambers were incubated with excess ascorbic acid oxidase (AAO), which catalyzes ascorbate oxidation to an electroinactive species. Then, the electrode chambers were analyzed by Pt-GOD as before; the results are in Table I. Now the outcome was as expected, with a higher glucose signal at the cathode than at the anode (by about a factor of three, which was in qualitative agreement with the experiments involving ethanol). It is noted that the incubation of the cathodal chamber solution with AAO did not appreciably alter the glucose signal detected by Pt-GOD, a result consistent with the fact that extraction of negatively charged ascorbate to the cathode was unlikely.

To confirm the validity of these findings, further experiments were performed in which the glucose concentration in the dermal bathing solution was varied. The results (Table II) show that, over the clinically relevant range of subdermal glucose concentrations (1–18 m*M*), a correlation between extracted level and perfused level was demonstrable. Glucose was even extracted when the dermal bathing solution concentration was set to zero. In this case, glucose already

Table I. Apparent Glucose Extraction (mean ± SD; $n = 10$) by Reverse Iontophoresis for 2 hr

	Extracted [glucose] (μ*M*)[a]	
	Pt/glucose oxidase	Modified copper electrode
Passive	0.3 ± 0.1	1.0 ± 0.1
Anode	20.0 ± 1.1	25.0 ± 1.4
Cathode	5.0 ± 0.8	10.0 ± 0.9
Anode (AAO)[b]	1.5 ± 0.2	nd[c]
Cathode (AAO)[b]	4.5 ± 0.2	nd

[a] Glucose concentration bathing the dermal skin surface was 5 m*M*.
[b] Results obtained when extracted sample was preincubated with ascorbic acid oxidase (AAO) prior to glucose biosensor analysis.
[c] Not done.

Table II. Glucose Extraction (mean ± SD; $n = 3$) at the Cathode by Reverse Iontophoresis[a] as a Function of the Subdermal Glucose Concentration

Subdermal [glucose] (mM)	Cathodal [glucose] (μM)[b]
1.0 ± 0.2	2.75 ± 0.20
5.0 ± 0.3	5.03 ± 0.30
18.0 ± 0.2	9.50 ± 0.20

[a] Current passed for 2 hr.
[b] Measured by the Pt-GOD sensor.

present in the epidermis and dermis is being extracted and measured. The radioactivity assay, however, was only able to measure radioactive glucose which was introduced in the dermal bathing solution: thus the measured lag times with this technique are anomalously high.

In conclusion, the results presented in this paper demonstrate that reverse iontophoresis, in conjunction with an appropriate analytical biosensor, has the potential to provide a novel, noninvasive, specific, and sensitive approach for glucose monitoring. Important outstanding questions pertain to (a) the *in vivo* acceptability and reproducibility of the method, (b) optimization of the kinetics of the extraction process, and (c) the responsiveness of the procedure to changes in blood glucose levels. In particular, while current has been passed for 2 hr in the experiments described here, a practical system must have a response time of the order of 10 min or less. Given that the amount extracted in 2 hr represents at least an order of magnitude more than the detection limit of our biosensor, we believe that practically feasible sampling times are possible. Even the most conservative estimates of glucose lag times would indicate that an operable system with sampling times $\leq$15 min can be realized. Finally, this work indicates that other molecules (i.e., ethanol and ascorbic acid) within and beneath the skin may also be amenable to transdermal monitoring applications in the same fashion.

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