

BIOSENSORS FOR CONTINUOUS IN VIVO GLUCOSE MONITORING

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ABSTRACT

A biosensor may be defined as a sensor incorporating a biological moiety, which can, without the addition of external reagents, determine the concentration of a specific analyte in tissue and body fluids. Based on this technology, we have developed a ferrocene-mediated amperometric glucose oxidase enzyme electrode for continuous *in vivo* measurement of subcutaneous tissue glucose in man. This sensor can be used to detect hypoglycaemia.

INTRODUCTION

The development of an implantable glucose sensor for continuous monitoring of glycaemic control in diabetic patients has been an important objective in bioengineering for several decades.

In conventional amperometric glucose sensors, the generated hydrogen peroxide [1] or consumed oxygen [2] due to the catalytic oxidation of glucose by immobilised glucose oxidase is measured. An alternative strategy is to incorporate a redox mediator such as ferrocene [3] into the sensor so that electrons are transferred directly from the prosthetic group of reduced glucose oxidase to a base electrode. The advantages of this approach include the fact that the reaction does not require the presence of molecular oxygen and is therefore less prone to changes in tissue oxygen tension. Also, because it is operated at low potential of 160 mV (c.f. 700 mV for hydrogen peroxide), there is less risk of interference from other electrochemically active compounds present in body tissues. We have previously reported the *in vitro* [4] and *in vivo* [5] characterisation of this technology. In this paper, we present preliminary results showing that mediated amperometric electrodes can be used to detect hypoglycaemia in man.

METHODS

The construction of the sensors has been described in detail [6] and a diagrammatic representation is shown in fig. 1.

The study group consisted of 6 fasting non-diabetic volunteers. A glucose sensor was implanted in the subcutaneous tissue on the anterior aspect of the forearm. A silver/silver chloride surface ECG electrode was used as a reference and placed on the skin close to the glucose sensor. Using a two electrode configuration, the glucose electrode was poised under potentiostatic control (Ministat model 251, Thompson Electrochem, Newcastle Upon Tyne, U.K.) at 160 mV and the output recorded on a chart recorder. All mains operated instruments were operated from an isolating transformer. A peripheral vein was cannulated for withdrawing blood samples for glucose determination on a YSI model 23 AM glucose analyser.

After an equilibration period of 20 minutes, 0.15 units/ Kg of short-acting insulin was injected intravenously so as to reduce the blood glucose concentration. Blood samples were taken every 5 minutes for 25 minutes and then 25 g of glucose (50 ml 50% w/v) solution was given intravenously to restore normoglycaemia.

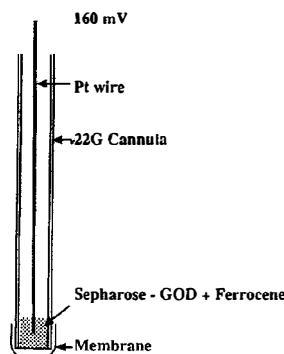


Fig. 1. Diagram showing construction of an *in vivo* glucose sensor.

RESULTS

We have made the assumption that under steady-state conditions (ie. at the beginning of the experiment - before insulin administration) the ratio of blood: tissue glucose concentration was unity. Tissue glucose concentrations (TG) were calculated using the equation:

$$TG = \frac{BG_n}{I_1} \times I_n$$

Where BG_n (mmol/l) and I_n (nA) are the blood glucose and current at time n, and I_1 (nA) is the initial current.

Fig. 2 shows the subcutaneous sensor and blood glucose concentrations in a representative normal subject given insulin to deliberately lower glucose values. Sensor values follow blood changes, though more slowly and reaching a nadir some 5 min after the lowest blood value.

DISCUSSION

Hypoglycaemia is a major clinical problem: it is probably the diabetic's greatest fear and often exceeds their concern about long-term tissue complications [7]. Twenty five percent of insulin-dependent diabetic patients suffer a serious hypoglycaemic attack about once a year requiring hospital attention [8] and it is the cause of death in 5% of diabetics who die under the age of 50 Y in the United Kingdom [9].

In this study we have demonstrated that it is possible to detect a fall in blood glucose concentration by means of an electrochemical sensor implanted in the subcutaneous tissue. Such a device may be developed as a useful hypoglycaemic alarm in diabetic patients.

We have opted for a single-point *in vivo* calibration in this study. A two-point *in vivo* calibration has been proposed by other workers [10] but would probably be impracticable for routine use.

In vitro studies have shown that the response time of the sensor is less than one minute and probably does not contribute significantly to the observed phase lag between the nadir for the blood and subcutaneous glucose. In the subjects we have studied to date, the mean delay was about three minutes but the range was 0 -15 minutes. Further studies are required to investigate whether the time lag remains constant within an individual or if not, which factors can cause a change.

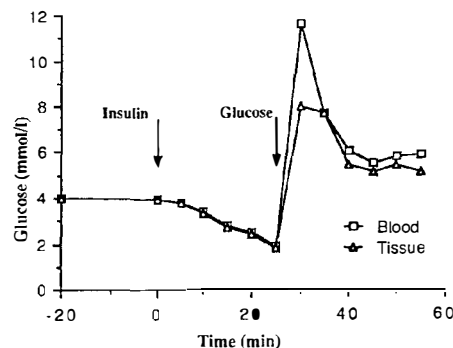


Fig. 2. Blood and tissue (sensor) glucose concentrations in a normal individual made hypoglycaemic.

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