Blood Glucose Regulation Using Closed- and Open-Loop Insulin Delivery Systems

II. Peripheral Primed Square Wave Infusions

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Summary. Glucose was infused into anaesthetized dogs before and after pancreatectomy. In the diabetics blood glucose was regulated first by closed-loop and then by open-loop insulin delivery schemes. Insulin requirements for the latter were determined by resolving the former into a sequence of 3 different infusion rates: during the baseline and recovery periods, basal insulin was delivered at 0.37 ± 0.02 mU/kg/min, while during the 60 min glucose infusion (10 mg/kg/min) there was an 8 min infusion at 4.96 ± 0.37 mU/kg/min and a 52 min component at 1.85 ± 0.08 mU/kg/min. With the open-loop method under these highly standardized conditions glycaemia was similar to normal controls but IRI levels were significantly higher, 13.5 vs 8.0 μ U/ml ($p < 0.05$) in the baseline and recovery periods and 74 vs 25 μ U/ml ($p < 0.05$) during the glucose infusion. It was concluded that: constant normoglycaemia can be maintained in the basal state by a constant rate of peripheral insulin delivery but at rates resulting in peripheral hyperinsulinaemia; the glycaemic response to glucose infusion can be normalized by a two component waveform of insulin delivery; and the closed-loop method can serve as a useful guide in determining insulin requirements.

Key words: Blood glucose regulation and control, glucose infusion, continuous low dose peripheral insulin infusion, pancreatectomized dogs, artificial beta cell, hyperinsulinaemia.

Studies using presently available closed-loop insulin delivery systems as an artificial endocrine pancreas [1, 2] have a number of limitations. Usually they are restricted in duration to one or two days, partly

because of the size of the device, but mainly because of the need for continuous blood withdrawal [3] for glucose sensing [4]. The glycaemic normalization achieved by these devices is frequently associated with peripheral hyperinsulinism [5–8] unless the insulin is delivered into the portal vein [9].

Because of these and other limitations several groups [7, 10–15] have described pre-programmed insulin delivery mechanisms. These open-loop devices do not depend on a glucose sensor and hence can be made portable enabling ambulant subjects to participate in studies lasting more than one or two days. Pre-programmed waveforms used included various diurnal combinations of a ‘basal’ insulin rate enhanced before or during the meal period either as one or more square waves [7, 10–12, 15] or in a pattern intended to reproduce the normal peripheral insulin profile [13]. Our group [7] studied pancreatectomized dogs for 12 months and showed that fasting euglycaemia and normal postprandial glucose levels could be obtained using a prototype portable insulin delivery device [14].

Insulin requirements before, during and after a caloric challenge can readily be determined by the application of a closed-loop system [5, 16, 17]. The primary aim of the present study was to examine whether a closed-loop waveform could be resolved into three simple open-loop components: a constant basal rate which would maintain normoglycaemia in the pre- and post-challenge periods and a combination of only two elevated rates which would normalize the glycaemic response to a peripheral glucose infusion. A secondary aim was to compare to normal the resulting IRI levels when peripheral insulin was delivered with the closed- and open-loop methods.

Materials and Methods

1. Animal Preparation and Blood Sampling

Six non-obese male Beagles, one to two years of age and weighing 11.3 ± 0.8 kg, were fasted for 12 h prior to each experiment. All were studied as controls but only five were included in the post pancreatectomy protocols. Dogs were fed a weight maintaining diet consisting of equal amounts of dog chow and canned food given once daily at 0900 h. General anaesthesia was induced with a IV 1:1 mixture of pentobarbital (Nembutal Sodium Injection, Abbott Laboratories, N. Chicago, Ill.) and pentothal (Pentothal Sodium, Abbott Laboratories, Montreal, Quebec) (25 mg/kg body weight). Anaesthesia was maintained throughout the experiment by a slow infusion (25 mg/h) of pentothal. A dual-lumen catheter (Abjad Industries, Mississauga, Ont.) was placed in each superficial jugular vein; one provided blood diluted 1:1 for continuous glucose monitoring and the other was arranged in such a way as to permit the withdrawal over short time intervals of essentially undiluted blood for the later determination of insulin. To compensate for blood lost during surgery and sampling 0.154 mol/l saline was infused at a rate of 1–2 ml/min throughout the experiment. Heparinized whole blood from a jugular vein catheter was collected at –40, –10, 0, 5, 10, 15, 30, 45, 60, 75, 90, 120, 150 and 180 min directly into 1½ ml centrifuge tubes, the start of the glucose challenge being designated time 0.

Blood was collected on ice and centrifuged with minimal delay at 4 °C, and supernatants frozen at –20 °C until assay.

2. Diabetes Induction and Management

Diabetes was induced by surgical removal of the pancreas as previously described [5, 17]. Subcutaneous injection of single doses of equal amounts of NPH and crystalline zinc porcine insulin formulations were given daily between experiments (Connaught Laboratories Ltd., Toronto, Ont.). The dose was based on fasting and 2 h postprandial blood glucose levels, determined 5 times weekly. Pancreatic enzyme supplementation (Cotazyme, Organon Ltd., Montreal, Quebec) was given with meals. All dogs maintained their presurgical weights (± 1 kg) with this treatment.

On the day prior to an experiment, the diabetic animals were given half the meal and half the dose of subcutaneous crystalline zinc insulin at 0900 and the remainder at 1700 h. Meals not finished by 2000 h were removed so that in all cases on the morning of the experiment the animals were hyperglycaemic (300–400 mg/dl), suggesting that very little residual insulin was present; concurrent plasma IRI levels were less than $2 \mu\text{U/ml}$. Euglycaemia was restored over 1–3 h by a combination of continuous intravenous infusion and boluses of 0.5 to 1.0 units insulin, and maintained for at least 40 min prior to glucose challenge.

3. Analytical Methods

A continuous blood glucose analyzer [18] was calibrated against a Beckman Glucose Analyser (Beckman Instruments, Fullerton, Cal.) to read equivalent plasma glucose concentration as previously described. Its total time delay was 2 min.

Plasma IRI was determined using an anti-porcine insulin antiserum (from Dr. Peter Wright, Minneapolis, Minn.), a dextran-coated charcoal separation of free from bound hormone, and a pork insulin standard (Novo Research Inst., Copenhagen, Denmark). Standard statistical methods were employed using the unpaired Student's *t* test to examine the significance of the differences between the responses of normal and diabetic animals. [All data are reported as mean $\pm$ SEM.]

Infused insulin was porcine neutral crystalline zinc insulin (Connaught Laboratories Ltd., Toronto, Ont.) diluted in 0.154 mol/l saline to a concentration of 17 mU/ml. The precision of the saline-insulin dilution was verified by immunoassay, as was the immunologic identity of the infused insulin and the standards in the assay conditions used (data not shown). Insulin infusion was with a pulsatile pump (Lambda Pump, Harvard Instruments Ltd., Millis, Mass.).

4. Blood Glucose Control

Glycaemic regulation in the diabetic dogs was realized first with closed-loop and then with open-loop methods, with an intervening interval of 7 to 28 days. The closed-loop method utilized the same continuous glucose analyzer as above in an artificial pancreas instrument [5, 17] in which the chosen algorithms [19] and constants were as defined in Appendix 1. Insulin was infused into a saphenous vein. With the closed-loop method the rate of insulin delivery responded on a minute-by-minute basis to both the blood glucose level and its rate of change.

With the open-loop method, the same pump was used and the rate of insulin delivery was fixed in a predetermined waveform with only 3 components. The selection of amplitudes and durations of these rates is described below.

5. Glucose Infusions

Glucose infusions consisted of a 60 min peripheral IV infusion into the contralateral saphenous vein at a rate of 10 mg/kg/min. All challenges started after a 40 min constant baseline period and were followed by a 120 min recovery period.

6. Waveform Determination for the Open-Loop Method

For each dog the rates of insulin delivery by the open-loop method were chosen to approximate the waveform obtained previously with the closed-loop method. Thus, during the baseline and recovery periods a constant basal rate of insulin was infused equal to the mean of that which was infused over the same periods using the closed-loop method. During the challenge period an initial high rate and a subsequent lower rate of insulin delivery were used, the former for 8 minutes and the latter for 52 minutes. The high rate was chosen on the basis of the maximum initial rate attained with the closed-loop method, while the lower rate was computed so that the total amount of insulin infused over the challenge was the same for both methods.

Results

1. Normal Controls (Figs. 1 and 2)

During glucose infusion blood glucose concentrations rose from baseline values of 98 ± 2 to peak values of 173 ± 7 mg/dl. When the infusion was terminated it dropped in 40 min to levels of 87 ± 2 mg/dl. The peripheral IRI concentrations were stable at $8 \pm 1 \mu\text{U/ml}$ during the 40 min baseline period and rose 3 fold during glucose infusion. Post-infusion IRI concentrations returned within 15 min to baseline values

Fig. 1. Glucose regulation before, during and after a peripheral glucose infusion of 10 mg/kg/min from 0 to 60 min. Normal controls ($n = 16$) and diabetic dogs ($n = 5$, closed circles) treated with a closed-loop insulin delivery system. Upper panel: glucose concentration – Centre panel: peripheral plasma insulin concentration – Lower panel: peripheral insulin infusion rates (diabetic dogs only)

and remained constant throughout the recovery period.

A total of 16 infusions were administered to the six normal dogs. Repeated studies under identical experimental conditions, either on the same day or weeks later, verified the reproducibility of the response of each dog. For example, one dog (12.8 kg) was studied 3 times, twice on one day and then again 30d later. With the first infusion blood glucose rose from baseline values of 103 to a peak value of 173 mg/dl while insulin levels rose from 12 to a peak of 23 μ U/ml. With the second infusion the corresponding values were from 91 to 169 mg/dl and from 11 to 25 μ U/ml. Thirty days later the corresponding values were from 96 to 165 mg/dl and from 6 to 25 μ U/ml.

2. Diabetic Dogs: Closed-Loop Insulin Infusion (Fig. 1)

During the baseline period the blood glucose concentration was 104 ± 4 mg/dl and rose in 20 min to a peak of 170 ± 7 mg/dl but declined during the final 40 minutes of the glucose infusion to 153 ± 10 mg/dl. Blood glucose then dropped rapidly to baseline levels and remained in this range throughout the recovery period.

Fig. 2. Glucose regulation before, during and after a peripheral glucose infusion of 10 mg/kg/min from 0 to 60 min. Normal controls (as in Fig. 1) and diabetic dogs ($n = 7$, open circles) treated with an open-loop insulin delivery system. Upper panel: blood glucose concentration – Centre panel: plasma insulin concentration – Lower panel: peripheral insulin infusion rates (diabetic dogs only)

Peripheral IRI levels in the baseline period were 15 ± 1 μ U/ml, rose 5–6 fold 15 minutes from the start of the challenge, and declined gradually thereafter until the infusion was terminated.

In the baseline period the mean insulin infusion rate was 4.2 ± 0.5 mU/min. During the glucose infusion the peak insulin infusion of 55 ± 6 mU/min occurred at 10 minutes, the rate declining rapidly by 30 minutes but remained elevated at 20 ± 2 mU/min. In the recovery period the insulin infusion declined rapidly to baseline values. The total amounts of insulin administered over the baseline, glucose infusion and recovery periods respectively were 169 ± 9 , 1534 ± 64 , 506 ± 26 mU.

3. Diabetic Dogs: Open-Loop Insulin Infusion (Fig. 2)

During the baseline period, the blood glucose concentration was 100 ± 4 mg/dl, the basal insulin infusion rate was 4.2 ± 0.2 mU/min and the peripheral IRI levels were 14 ± 1 μ U/ml. Glucose levels rose to a peak of 155 ± 13 mg/dl during the infusion and fell to preinfusion levels within 20 min after the termination of the glucose infusion. Peripheral plasma IRI levels rose to a peak of 74 ± 16 μ U/ml in 5 min and then declined to a plateau of 40 ± 5 μ U/ml during

the remainder of the glucose infusion. In the recovery period IRI levels returned to steady baseline values in approximately 15 minutes.

The insulin infusion rate in both the baseline and recovery periods was constant at 4.2 ± 0.2 mU/min. During the glucose infusion the waveform included an initial high rate of 56 ± 4 mU/min for 8 minutes and then a rate of 21 ± 1 mU/min for 52 min. Total amounts of insulin infused during the baseline, glucose infusion and recovery periods were respectively 183 ± 14 , 1620 ± 165 , and 466 ± 62 mU.

4. Diabetics versus Controls

Data in Figure 1 compare the normal controls and the diabetics treated with the closed-loop system. Glycaemia differed significantly ($p < 0.015$) from controls only at 10 minutes when it was higher by 20 mg/dl. Peripheral IRI levels were significantly higher than controls at all times. At 75 minutes these differences were not significant ($p > 0.05$).

With the open-loop method of treatment (Fig. 2) glycaemia differed from controls only at 30 minutes when it was 28 mg/dl lower. Peripheral IRI levels were significantly higher than controls at all times except at 75 minutes when these differences were not significant. During the course of the experiment, the insulin infusion rates (Fig. 2) differed only at times 1, 10 and 15 minutes; however, the same total amounts of insulin were infused in the baseline, challenge and recovery periods.

Discussion

Closed-loop systems normalize glycaemia and provide information of the minute-by-minute insulin requirements for a variety of caloric challenges. This information has been useful in determining and adjusting subcutaneous insulin requirements [20]. In this report we demonstrate that it can be used in programming open-loop insulin delivery devices. To this end, the insulin requirements to a glucose infusion were determined in dogs using the closed-loop method. After each experiment the resulting insulin infusion pattern was abstracted into a 3-component waveform for use in subsequent open-loop studies on the same dog. For the group this procedure resulted in an insulin infusion waveform which was remarkably comparable for both methods (Figs. 1 and 2, lower panels) and equal amounts of insulin were infused during the glucose infusion and of course during the baseline and recovery periods when the basal rate for the open-loop method is easily chosen from the closed-loop data.

In the open-loop study, the initial high rate of insulin delivery coincided with the start of the glucose

infusion. The combination of the high rate of insulin delivery and the lack of delay in its initiation resulted in a clear attenuation of the glycaemic rise with the open-loop waveform. Glycaemia was thus significantly ($p < 0.05$) lower than controls at 20 min (Fig. 2, upper panel), the difference disappearing by the end of the challenge. IRI levels in the diabetics (Figs. 1 and 2) were significantly higher than controls at all times. Thus glycaemic normalization in anaesthetized dogs is accompanied by peripheral hyperinsulinism when the insulin is infused peripherally. This does not occur under similar experimental conditions when insulin is delivered portally [9] although it has been reported following pancreatic islet transplantation in the peritoneum of diabetic rats [21]. The results presented here support the contention [22] that minimizing the delay of the closed-loop system probably reduces the required amount of early insulin. In the present study the closed-loop instrument had a delay of 2 minutes and included parameters (Appendix 1) which accelerated insulin delivery some 11 fold with rising glycaemia (Fig. 1). Perhaps a greater than necessary amount of insulin was given with the similar but earlier high infusion rate of the open-loop study because it was applied without delay. Resulting glycaemic levels were lower than normal as shown in Figure 2.

In these studies the 60 minute glucose infusion at a rate of 10 mg/kg/min into a peripheral vein was chosen because it is a defined caloric challenge which resulted in reproducible glucose and insulin responses in the anaesthetized dog. We have observed no differences in the responses of diabetic dogs when the glucose infusion was delivered portally [5, 9] or peripherally [17]. The latter route was used in this study to explore the adequacy of this route (the only one feasible in human studies at present), and to avoid the necessity of repeated laparotomy. This intravenous glucose infusion in pancreatectomized dogs produced a glycaemic response which previously could only be normalized by a closed-loop insulin delivery system specifically programmed to meet this challenge.

The major advantage of the open-loop system is its simplicity, applicability and the elimination of the need for a glucose sensor. Within the constraints of the present study, we conclude that preprogramming of open-loop waveforms can be guided by the results of closed-loop studies. Whether these findings can be extended to the heterogeneous situation of human diabetes mellitus remains to be established.

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Appendix I

The algorithms for the closed-loop control system are as follows:

$$R_i = \frac{1}{2} M_i [1 + \tanh S_i (G_p - B_i)]$$

$$G_p = G + DF$$

$$DF = K_1 (A)^3 + K_2 (A)$$

where,

G_p is the 'projected' glycaemia in mg/dl

G is the measured glycaemia in mg/dl

R_i is the rate of insulin delivery in mU/min

M_i is the maximum rate of insulin delivery in mU/min

$\tanh$ is the hyperbolic tangent function which is defined

$$\tanh(x) = [\exp(x) - \exp(-x)] / [\exp(x) + \exp(-x)]$$

S_i is the steepness parameter of the argument of the hyperbolic tangent function in dl/mg

B_i is the position parameter in the argument of the $\tanh$ function in mg/dl and corresponds to the glycaemia at which the half-maximum insulin infusion rate occurs. Changing B_i shifts the curve to the left or right without changing its shape.

DF is the difference factor in mg/dl and depends on the rate of change of glycaemia

K_1 is the scaling parameter for the cubic term in DF

K_2 is the scaling parameter of the linear term in DF

A is the linearly weighted mean time rate of change of glycaemia in mg/dl/min computed each minute on the basis of the present (G_0) and previous three (G_{-1} , G_{-2} , G_{-3}) measured glycaemias.

$$A = (4 G_0 - 3 G_{-1} - 2 G_{-2} + G_{-3}) / 10$$

The following parameters were used throughout:

$$M_i = 0.50 \text{ mU/kg/min}$$

$$K_1 = 1 \text{ (mg/dl)}^{-2}$$

$$S_i = 0.0175 \text{ dl/mg}$$

$$K_2 = 10 \text{ (mg/dl)}$$

$$B_i = 175 \text{ mg/dl}$$

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