Indirect evidence for short-loop negative feedback of insulin secretion in the rat

H. P. T. Ammon, C. Reiber and E. J. Verspohl

Department of Pharmacology, Institute of Pharmaceutical Sciences, University of Tübingen, Auf der Morgenstelle 8, D-7400 Tübingen, Federal Republic of Germany

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ABSTRACT

Feedback inhibition of glucose-mediated insulin release has repeatedly been demonstrated in isolated pancreatic islets and in the perfused pancreas. It was the aim of the present study to determine whether inhibition occurs through a long-loop (plasma concentration of insulin) or a short-loop (local concentration) action of insulin. The perfused rat pancreas was used, with different perfusion rates and different insulin concentrations in the medium. Increasing the flow rate from 1 to either 3 or 6 ml/min gradually decreased the insulin concentration in the effluent, at stimulatory concentrations of glucose (11.1 and 16.7 mmol/l). Under the same conditions, however, the integrated amount of insulin released over a period of 30 min was significantly enhanced. When exogenous insulin (2.7 and 5.4 μmol/l) was added to the perfusion medium, insulin secretion in the presence of 11.1 or 16.7 mmol glucose/l at flow rates of 3 and 6 ml/min was diminished. This effect was most prominent with 11.1 mmol glucose/l and 2.7 μmol exogenous insulin/l at all flow rates (except 1 ml/min), as well as at the high perfusion flow rates with other glucose concentrations. Insulin secretion was not affected by 5.4 μmol exogenous insulin/l at 1 ml/min or by 2.7 μmol exogenous insulin/l at 3 ml/min. The data support a negative feedback inhibition of insulin secretion by secreted insulin, since insulin secretion was decreased by either adding exogenous insulin or by lowering endogenous insulin as the consequence of increased flow rates. They also suggest that the local extracellular concentration of insulin is of more importance than the plasma concentration, consistent with the concept of a short-loop feedback as already claimed for other hormones and/or neurotransmitters. Journal of Endocrinology (1991) 128, 27–34

INTRODUCTION

There is evidence that secretion of insulin is under negative feedback control both in isolated pancreatic islets as well as in the perfused pancreas. This was assumed since exogenous insulin diminishes the secretory response of insulin to glucose (Sodoyez, Sodoyez-Goffaux & Foà, 1969; Iversen & Miles, 1971; Ammon & Verspohl, 1976; Verspohl, Händel, Hagenloh & Ammon, 1982; Verspohl & Ammon, 1983) and since insulin antibodies, by inactivating freshly released insulin, augment glucose-mediated insulin secretion (Ziegler, Hahn & Klat, 1972; Loreti, Dunbar, Chen & Foà, 1974; Verspohl, Händel & Ammon, 1979). There have been contradictory results from in-vivo studies employing euglycaemic or hyperglycaemic clamps (Lilienquist, Horwitz, Jennings et al. 1978; Greenfield, Doberne, Kraemer et al. 1981; Waldhäusl, Gasić, Bratusch-Marrain et al. 1982; Bratusch-Marrain & Waldhäusl, 1985; Argoud, Schade & Eaton, 1987). In vitro, a negative feedback was not confirmed by several groups (Grodky, Curry, Bennett et al. 1968; Schatz & Pfeiffer, 1976; Tanaka, Shima, Sawazaki et al. 1980).

Feedback control of hormone and/or neurotransmitter release is generally believed to occur through long-loop feedback (plasma concentration) or short-loop feedback (concentration of released hormones close to the secreting cell). It is difficult to detect short-loop feedback by using in-vivo experiments since the hormone concentration present in the plasma may not be representative of the concentration around the secretory cell. Hellman & Lernmark (1969) pointed out that insulin release from islet B cells could be locally regulated and proposed a correlation between blood flow and insulin secretion. It has further been shown that pancreatic and islet blood flow is regulated by the glucose concentration in vivo (Vetterlein, Meyer, Reitemeyer et al. 1981; Jansson & Hellerström, 1983; Jansson, 1984; Qamar, Read & Mountford, 1986). An
indirect approach to study whether feedback in a perfused system represents a short- or long-loop system is to change the hormone concentrations around the secretory cells by variation of blood flow rates and/or by adding hormone to the perfusion medium. We therefore set out to study the effect of different flow rates with different insulin concentrations on glucose-mediated secretion of insulin from the perfused rat pancreas.

MATERIALS AND METHODS

Animals

Adult Wistar rats of either sex weighing between 230 and 330 g were used. They were maintained at 22 °C with a 12 h light:12 h darkness cycle, and a standard pellet diet (Fa. Altromin, Lage/Lippe, F.R.G.) and tap water were available ad libitum.

Chemicals

α-d-glucose and dextran 60 were obtained from Serva Feinbiochemica, Heidelberg, F.R.G., bovine serum albumin (BSA) fraction V was from Behringwerke AG, Marburg, F.R.G., and hexobarbitone-Na (Evipan) was from Bayer, Leverkusen, F.R.G. L-lactate tests were purchased from Boehringer, Mannheim, F.R.G., and the rat insulin from Novo Research Institute, Copenhagen, Denmark. Insulin radioimmunoassay kits (INSIK-I) were supplied by Isotopendienst West, Dreieich, F.R.G. All other chemicals and reagents of analytical grade were from E. Merck, Darmstadt, F.R.G.

Perfusion studies

Rats were anaesthetized by an i.p. injection of hexobarbitone-Na (15 mg/100 g body weight), and the pancreata isolated by the technique described by Curry, Bennett & Grodsky (1968) (perfusion through arteria coeliaca).

The perfusion medium was a Krebs–Ringer-bicarbonate buffer supplemented with 4% (w/v) dextran and 0-25% (w/v) BSA and gassed with 95% O₂ plus 5% CO₂ to maintain a final pH of 7-4. The basal medium contained 2-8 mmol glucose/l and the test medium 11-1 or 16-7 mmol glucose/l. The medium reservoirs were maintained at 37 °C and the perfusion mediums were not recycled.

During the first 15 min after the isolation procedure the perfusion flow rate was adjusted to 1, 3 or 6 ml/min by varying the velocity of the peristaltic pump. In the next 15 min the pancreas was allowed to equilibrate under constant conditions of flow rate and pressure. Perfusion pressures were determined by a simple manometer in the form of a long open glass tube and expressed as cm perfusion medium.

In the following 5 min (−5 to 0 min) five samples were collected to determine basal insulin secretion. At time 0, the test medium, e.g. containing exogenous insulin (2-7 or 5-4 μmol/l), was introduced. Exogenous insulin was added to the test medium immediately before changing the medium reservoir. During the stimulation period of 30 min, eight samples were taken at 1-min intervals and a further 11 samples at 2-min intervals for the assay of insulin. In addition, samples were taken at 5-min intervals from both the basal and the test medium reservoirs in order to determine the amount of exogenous insulin.

The venous effluent was collected in graduated tubes to determine the perfusion flow rate.

Assay of insulin

Insulin concentrations in the effluent and the reservoir were measured in duplicate by a double-antibody method using the INSIK-I radioimmunoassay kit and rat insulin as a standard.

Recovery of exogenous insulin (determination of insulin extraction and degradation)

Recovery of exogenous insulin in the effluent and its degradation in the gassed (95% O₂ plus 5% CO₂) medium reservoirs were checked by performing perfusion experiments in the absence of glucose. Recovery of 2-7 and 5-4 μmol exogenous insulin/l at flow rates of 1, 3 and 6 ml/min is shown in Fig. 1; exogenous insulin was added in the preperfusion and the main perfusion period, the concentration and s.e. of which is shown by the shaded areas. The drawn lines represent the insulin concentrations in the effluent. Extraction or retention of insulin by the pancreas was only significant (P<0-05) at the lowest flow rate (Table 1). The results of subsequent experiments carried out under the same conditions were corrected for the percentage of extracted insulin except in the studies using a rate of 6 ml/min.

Viability of the pancreas preparation

Impairment of pancreatic function caused by insufficient oxygen supply at low perfusion flow rates could produce false results, since diminished insulin secretion at low flow rates could be the result of cell damage rather than of increased feedback inhibition. To exclude the possibility of a deficient oxygen supply, the secretion of L-lactate from the perfused

Effects of perfusion flow rate and pressure on glucose-stimulated insulin secretion

The perfusion parameters of flow rate and pressure are usually interdependent; an increase of the perfusion flow rate provokes a concomitant rise in perfusion pressure. When a variation in flow rate causes a change in the rate of insulin secretion, it cannot be determined whether this is due to the actual change in flow rate, to the change in pressure, or to both. To elucidate the influence of both these parameters on insulin secretion from the perfused pancreas, the venous effluent tube was fitted with a clamp in order to be able to increase the vascular resistance, so enabling perfusion studies to be performed at different flow rates under isobaric conditions. In studies using flow rates of 1 and 3 ml/min under artificially increased pressure conditions, the perfusion pressure was adjusted to that of the next highest flow rate. Basal values of insulin concentration were obtained at the end of the experiments when 3 mmol glucose/l was used (control; data not shown).

Calculation of results

Insulin concentration (µmol/l) multiplied by the perfusion flow rate (ml/min) gave the amount of insulin at each time-interval (µmol/min). The integrated amount of secreted insulin (area under the curve; AUC) was calculated by adding the secreted amounts over 30 min and subtracting the basal amount of secreted insulin. In perfusion experiments using exogenous insulin the integrated exogenous insulin was subtracted from the AUC.

Results are shown as means ± s.e. For statistical evaluation multiple comparisons of means were carried out by two-way analysis of variance (F-ratio analysis - not shown).

TABLE 1. Recovery of exogenous insulin in the effluent. Perfusion experiments with rat pancreata were performed in the presence of 2.7 and 5.4 µmol exogenous insulin/l without glucose stimulation at various perfusion flow rates. Values are means ± s.e., n = 8

<table>
<thead>
<tr>
<th>Perfusion flow rate (ml/min)</th>
<th>Concentration of exogenous insulin (µmol/l)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.7</td>
<td>62±11</td>
</tr>
<tr>
<td>1</td>
<td>5.4</td>
<td>85±5</td>
</tr>
<tr>
<td>3</td>
<td>2.7</td>
<td>98±1</td>
</tr>
<tr>
<td>3</td>
<td>5.4</td>
<td>96±4</td>
</tr>
<tr>
<td>6</td>
<td>2.7</td>
<td>101±1</td>
</tr>
<tr>
<td>6</td>
<td>5.4</td>
<td>98±2</td>
</tr>
</tbody>
</table>

pancreas stimulated with 16·7 mmol glucose/l over 35 min at flow rates of 1, 3 and 6 ml/min was determined. There was no evidence for oedema of the pancreas preparation at any of the perfusion rates. Motility of the duodenum was observed throughout the experiments.

FIGURE 1. Recovery of exogenous insulin after perfusion of isolated rat pancreas. Perfusion experiments with rat pancreata were performed in the presence of 2.7 (top graph of each pair) and 5.4 µmol exogenous rat insulin/l (bottom graph of each pair) without glucose stimulation at perfusion flow rates of (a) 1, (b) 3 and (c) 6 ml/min. Results are shown as insulin concentrations reaching the pancreas (shaded areas; measured every 5 min starting from zero min) and recovered from the effluent (solid lines). The medium was changed to an identical medium at time 0 as indicated by the arrows at the top. Values are the mean ± s.e., n = number of experiments.
RESULTS

At flow rates of 1, 3 and 6 ml/min the insulin concentration in the effluent, at 11·1 and 16·7 mmol glucose/l, exhibited the typical biphasic pattern (Fig. 2). The

insulin concentration in the effluent (µmol/l) decreased with increasing flow rates (Fig. 2). However, when secreted insulin was calculated as the total amount over 30 min (AUC), higher values for insulin secretion were found at flow rates of 3 and 6 ml/min than at a low flow rate of 1 ml/min (Fig. 3).

Effect of flow rate and exogenous insulin on insulin secretion

At various flow rates, glucose concentrations of 11·1 and 16·7 mmol/l were combined with 2·7 and/or 5·4 µmol exogenous insulin/l as 2·7 and 5·4 µmol/l are in the range of the physiological insulin concentrations released by the isolated perfused pancreas (5·73, 3·17 and 2·36 µmol/l at flow rates of 1, 3 and 6 ml/min respectively). Also, according to Sodoyez et al. (1969), the rate of insulin secretion depends on both the stimulator glucose and the inhibitor insulin. High concentrations of exogenous insulin were therefore expected to be necessary for the inhibition of insulin secretion when mediated by a highly increased glucose concentration. The maximal insulinotropic glucose concentration (16·7 mmol/l) was therefore combined with 2·7 and 5·4 µmol exogenous insulin/l. At 1 ml/min, 2·7 µmol/l was not used since this concentration was expected to have insufficient inhibitory potency in this perfusion system. At 6 ml/min the insulin concentration of 5·4 µmol/l was omitted in order to avoid difficulties of calculation because of the high concentration of exogenous insulin compared with the estimated low concentration (approximately 2·0 µmol/l) of secreted insulin. Using a half-maximal insulinotropic glucose concentration (11·1 mmol/l), the concentration of 2·7 µmol exogenous insulin/l was expected to produce sufficient inhibition of insulin secretion at all flow rates.
The effects of exogenous insulin at various flow rates on insulin release stimulated by both 16.7 and 11.1 mmol glucose/l are shown in Figs 4 and 5 respectively. Perfusion with 5.4 μmol insulin/l at 1 ml/min and 2.7 μmol insulin/l at 3 ml/min did not affect insulin secretion, whereas 5.4 μmol/l at 3 ml/min and 2.7 μmol/l at 6 ml/min significantly inhibited insulin secretion. During stimulation with 11.1 mmol glucose/l, 2.7 μmol exogenous insulin/l inhibited insulin release at both the higher flow rates. The inhibitory effects of exogenous insulin were most obvious at the lower glucose concentration and at the increased flow rates of 3 and 6 ml/min (Table 2).

Viability of the pancreas preparation

Different perfusion flow rates had no effect on the L-lactate secretion of the pancreas except the low flow rate of 1 ml/min. L-lactate production at 1 ml/min was significantly lower (4.107 ± 0.0338 mg/35 min) than that at the higher flow rates (3 ml/min, 5.5064 ± 0.161; 6 ml/min, 4.975 ± 0.307 mg/35 min, n = 3). It was, therefore, supposed that all perfusion flow rates used, even the lowest, provided enough oxygen for the pancreatic tissue.

Effects of the perfusion flow rate and pressure on glucose-stimulated insulin secretion

Table 3 shows the effect of both perfusion flow rate and pressure on the glucose (16.7 mmol/l)-induced insulin secretion. An artificially increased perfusion pressure resulted in slightly decreased insulin secretion, which was found to be significant only at a flow rate of 1 ml/min. An increase in perfusion flow rate produced a significant increase in insulin release. This effect was more likely to be due to the flow rate than to the effects of pressure which were negligible.

DISCUSSION

Relationships between perfusion flow rate and hormone release, which are thought to be correlated with changes in hormone autofeedback, have been reported from a variety of isolated perfused organs, such as the rat heart (Fuder, Barth, Wiebelt & Muscholl, 1984), the rat adrenal (Hinson, Vinson, Whitehouse & Price, 1986; Hinson, Vinson & Whitehouse, 1986) and the human pituitary gland (Boscaro, Sonino, Paoletta et al. 1988). Using the isolated perfused pancreas, relationships between flow rate and exocrine secretory function have been observed (Augier, Boucard, Pascal et al. 1972). With respect to endocrine secretion, Grodsky et al. (1968) did not observe any effect of a rapid decrease in perfusion flow rate on total insulin secretion. The influence of various perfusion flow rates on insulin release has not, however, been elucidated in detail.

The data in this study clearly demonstrate that by increasing flow rates at both 11.1 and 16.7 mmol glucose/l the insulin concentration in the effluent was decreased whereas total insulin secretion calculated as AUC over 30 min was significantly increased.
TABLE 2. Inhibitory effect of 2·7 and 5·4 μmol insulin/l on insulin release at various flow rates and glucose concentrations. Rat pancreata were perfused with Krebs–Ringer-bicarbonate buffer, supplemented with dextran (4%), bovine serum albumin (0·25%), and gassed with 95% O₂ : 5% CO₂. The inhibitory effects of exogenous insulin were calculated in relation to the corresponding control experiment without exogenous insulin, using the mean values of the insulin secretion. Values are means ± s.e., n = 8

<table>
<thead>
<tr>
<th>Perfusion flow rate (ml/min)</th>
<th>Glucose concentration (mmol/l)</th>
<th>Concentration of exogenous insulin (μmol/l)</th>
<th>Inhibitory effect by (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16·7</td>
<td>5·4</td>
<td>15 ± 8·1</td>
</tr>
<tr>
<td>3</td>
<td>16·7</td>
<td>2·7</td>
<td>14 ± 5·4</td>
</tr>
<tr>
<td>3</td>
<td>16·7</td>
<td>5·4</td>
<td>52 ± 9·3*</td>
</tr>
<tr>
<td>6</td>
<td>16·7</td>
<td>2·7</td>
<td>30 ± 11*</td>
</tr>
<tr>
<td>6</td>
<td>11·1</td>
<td>2·7</td>
<td>25 ± 8·6*</td>
</tr>
<tr>
<td>6</td>
<td>11·1</td>
<td>2·7</td>
<td>37 ± 6·5*</td>
</tr>
</tbody>
</table>

*P < 0·001 compared with control (no exogenous insulin) (Student’s t-test after two-way ANOVA (F-ratio test)).

TABLE 3. Effect of perfusion flow rate and perfusion pressure on the 16·7 mmol glucose/l-induced insulin secretion from isolated rat pancreas. Rat pancreata were perfused with Krebs–Ringer bicarbonate buffer, supplemented with dextran (4%), bovine serum albumin (0·25%), and gassed with 95% O₂ : 5% CO₂. The artificial increase in perfusion pressures was obtained by constricting the venous effluent. Insulin secretion was calculated as area under the curve (AUC) for the perfusion test period of 30 min. Values are means ± S.E.M. for the number of experiments in parentheses

<table>
<thead>
<tr>
<th>Perfusion pressure</th>
<th>Flow rate (ml/min)</th>
<th>Perfusion pressure (cm medium)</th>
<th>Perfusion flow rate (ml/min)</th>
<th>Insulin secretion (AUC; μmol/30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual (5)</td>
<td>1</td>
<td>38 ± 1·5</td>
<td>1·0 ± 0·02</td>
<td>153·8 ± 17·29</td>
</tr>
<tr>
<td>Increased (4)</td>
<td>1</td>
<td>71 ± 1·0</td>
<td>1·0 ± 0·02</td>
<td>979·6 ± 34·49</td>
</tr>
<tr>
<td>Usual (5)</td>
<td>3</td>
<td>73 ± 3·3</td>
<td>3·1 ± 0·1</td>
<td>249·7 ± 37·39*</td>
</tr>
<tr>
<td>Increased (5)</td>
<td>3</td>
<td>113 ± 3·5</td>
<td>2·9 ± 0·05</td>
<td>222·1 ± 47·19</td>
</tr>
<tr>
<td>Usual (5)</td>
<td>6</td>
<td>112 ± 3·0</td>
<td>5·9 ± 0·1</td>
<td>426·6 ± 45·99††</td>
</tr>
</tbody>
</table>

*P < 0·05 compared with usual pressure at 1 ml/min; ††P < 0·02 compared with usual pressure at 3 ml/min (Student’s t-test after two-way ANOVA (F-ratio test)).

lower concentration of insulin in the medium may be an indirect indication that the concentration of insulin around the secretory cells is reduced, and it is logical to assume that this is due to the high flow rate. On the other hand, the fact that the overall amount of insulin secreted during stimulation with glucose was markedly increased provides indirect evidence for a decreased feedback inhibition which may be the consequence of a diminished insulin concentration in the extracellular space at high flow rates. In contrast, under conditions of low flow rates when freshly secreted insulin is removed more slowly, higher concentrations of insulin are probably present around the B cell, thus accounting for the lower overall amount of insulin secreted during glucose stimulation because of the inhibitory action of recently released insulin.

A dependence of insulin secretion upon the balance between a positive (glucose concentration) and a negative (insulin concentration) insulinogenic stimulus, has already been suggested by Sodoyez et al.
insulin in the immediate vicinity of the B cells is important for controlling the degree of inhibition, probably through a short-loop feedback mechanism.

REFERENCES


secretion by exogenous insulin in normal man as demonstrated by C-peptide assay. *Diabetes* 27, 563–570.


