Different dynamics of insulin secretion in the perfused pancreas of mouse and rat

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Abstract. The dynamics of insulin release were studied in the perfused pancreas of rats and mice. Perfusion of the rat pancreas with 20 mM D-glucose resulted in the classical biphasic release of insulin with a rising second phase. However, in normal C57BL/KsJ-mice and non-inbred mice, whether fed or starved, the second phase was nearly constant. The secretory dynamics of KsJ-mice were essentially the same, whether the glucose concentration was 30 or 20 mM, whether the medium contained 2.56 or 8 mM Ca\(^{2+}\), and whether or not the medium was supplemented with 5 mM pyruvate, 5 mM glutamate, and 5 mM fumarate. Insulin secretion in these mice was almost totally inhibited by omission of Ca\(^{2+}\), and was markedly enhanced by 3-isobutyl-1-methylxanthine. Insulin release during the constant phase was reversed by lowering the glucose concentration. A second rise of glucose from 3 to 20 mM produced a secretory pattern very similar to the first response. These studies indicate that the dynamics of insulin secretion are somewhat different in rats and mice. Since similar results were obtained with C57BL/KsJ-mice and non-inbred mice, the liability of KsJ-mice to develop β-cell failure when stressed by the mutated db gene is not related to the constancy of the second insulin secretory phase.

In several animal species insulin release from the perfused pancreas is known to be biphasic when stimulated by a constant concentration of D-glucose (Grodsky et al. 1967; Curry et al. 1968, 1975; Bennett & Grodsky 1969; Iversen 1971; Frankel et al. 1974). Thus in rats, the most commonly employed animal model, the dynamics of insulin release is characterized by a sharp first spike followed by a second phase of more slowly increasing secretory rate (Curry et al. 1968). In the more seldom investigated Syrian hamster, however, the second phase is represented by a more constant rate of secretion (Curry et al. 1975). The secretory pattern in mice is unclear. In this species too, only a few studies of the perfused pancreas have been performed. According to Laube et al. (1973), the dynamics of insulin release in mice is very similar to that in the rat. However, Berglund et al. (1978) did not find a rising second phase when perfusing the pancreas of normal C57BL/KsJ-mice.

Mice with the C57BL/KsJ genetic background have a disposition to develop a severe and insulin-dependent diabetes when carrying the gene, db, in homozygous form. On other genetic backgrounds, db causes only a mild form of diabetes without B-cell degeneration (Boquist et al. 1974). It is therefore important to know whether the flat second phase of insulin release previously reported for normal C57BL/KsJ-mice, is specific for this type of mice and hence may be an aberration related to the diabetogenic disposition.

In the present study the dynamics of insulin release in non-diabetic C57BL/KsJ-mice are compared with those in non-inbred mice as well as Sprague-Dawley rats.

Materials and Methods

Animals

Non-diabetic C57BL/KsJ-mice, 24–30 weeks old, and non-inbred mice of the same age were taken from local colonies. The non-inbred mice were phenotypically normal individuals from a stock carrying the gene ob; they either lacked this gene or had it in heterozygous form only.

Sprague-Dawley rats, 9 months old, were purchased from Anticimex AB, Stockholm, Sweden. Animals of both sexes were used, and unless otherwise stated they were starved for 18 h before being dissected.
**Pancreas perfusion**

As previously (Berglund et al. 1978), a modification of the technique described by Grodsky & Fanska (1975) was used. The basal perfusion medium was Krebs-Ringer bicarbonate buffer (De Luca et al. 1964), which had been filtered through a Millipore filter (type GS, 0.22 μm pore size). The medium was supplemented with 40 mg/ml bovine serum albumin fraction V (Sigma Chemical Co., St. Louis, MO), gassed with O₂–CO₂ (95:5) and warmed to 37°C for 1 h before the perfusion started. pO₂ was 73.3–77.3 kPa and pH was 7.4 when the medium reached the pancreas. The flow rate, 0.4–0.6 ml/min for mouse pancreas and 6 ml/min for rat pancreas, was adjusted to give a perfusion pressure of 6.67 ± 0.67 kPa in all experiments. During the perfusion the pancreas was generally placed on a dry plate kept at 37°C. In a few control experiments, however, the pancreas was surrounded by basal medium of the same temperature. The buffer was modified in 5 different ways to characterize the dynamics of insulin release as described in the Results section.

Each experiment was begun by perfusing the pancreas for 30 min with basal medium containing 3 mM D-glucose to stabilize the basal secretory rate.

**Assay of insulin and glucose**

Insulin was assayed radioimmunologically with 125I-labelled pig insulin (Farbwerke Hoechst AG, Frankfurt/M., Germany) and with crystalline mouse insulin as standard. Free and antibody bound insulin were separated by precipitation with 81% (v/v) ethanol (Heding 1966). The albumin content of the phosphate buffer was 40 mg/ml. Samples of perfusate were analyzed for glucose with the coupled hexokinase-glucose-6-phosphate dehydrogenase method (Lowry et al. 1964).

**Results**

Perfusion of the rat pancreas with 20 mM D-glucose resulted in a biphasic release of insulin with a rising second phase (Fig. 1). When the pancreas of KsJ-mice were perfused with 20 mM D-glucose, a distinct first phase of insulin release occurred. However, the second phase was characterized by a nearly constant secretory rate for more than 30 min (Fig. 2). The same pattern of secretory dynamics was recorded with the pancreas of non-inbred mice (Fig. 2). Similar results were obtained with KsJ-mice and non-inbred mice which had not been starved overnight (not shown).

The fairly constant second phase of the secretory dynamics was essentially the same whether the KsJ-mouse pancreas rested on a dry plate or was immersed in buffer with a temperature of 37°C.

![Fig. 1.](image)

Mean insulin release during perfusion of pancreas from 3 Sprague-Dawley rats. After 30 min of pre-perfusion with 3 mM D-glucose the glucose concentration was suddenly increased to 20 mM and kept at this concentration during the subsequent 45 min. The abscissa shows min after changing the glucose concentration. The ordinate shows rate of insulin release. The basal release 3 min before exposure to 20 mM glucose was 0.9 ± 0.7 ng/min, the peak response to 20 mM glucose was 95.3 ± 64.2 ng/min, and the release during min 20–25 was 190.0 ± 107.9 ng/min (mean values ± SEM). The dotted line shows the glucose concentration as a function of time.
Fig. 2.
Mean insulin release during perfusion of pancreas from 3 non-inbred lean mice (open circles), and 3 normal C57BL/KsJ-mice (solid circles). Solid squares show the mean insulin release from 3 normal C57BL/KsJ-mice, when Ca\(^{2+}\) is omitted. Solid triangles show the glucose concentration as a function of time. The basal release 3 min before exposure to 20 mM glucose was 0.3 ± 0.1 ng/min for non-inbred lean mice, 0.6 ± 0.6 ng/min for normal C57BL/KsJ-mice, and 0.1 ± 0.1 ng/min for normal C57BL/KsJ-mice when Ca\(^{2+}\) was omitted from the medium. The peak values were 38.1 ± 23.7 ng/min for non-inbred lean mice, 23.1 ± 10.7 ng/min for normal C57BL/KsJ-mice, and 1.5 ± 1.3 ng/min for normal C57BL/KsJ-mice when Ca\(^{2+}\) was omitted from the medium. The release during min 20–25 was 1.9 ± 0.7 ng/min for non-inbred lean mice, 4.2 ± 1.6 ng/min for normal C57BL/KsJ-mice, and 0.3 ± 0.1 ng/min, when Ca\(^{2+}\) was omitted from the medium (mean values ± SEM).

Fig. 3.
Mean insulin release during perfusion of pancreas from 4 normal C57BL/KsJ-mice with a high calcium concentration. Both during the 30 min of pre-perfusion with 3 mM D-glucose and the subsequent 45 min of perfusion with 20 mM D-glucose, the calcium concentration was kept at 8 mM. Glucose concentration was changed at time zero on the abscissa. The basal release 3 min before exposure to 20 mM glucose was 0.1 ± 0.1 ng/min, the peak response to 20 mM glucose was 95.3 ± 64.2 ng/min, and the release during min 20–25 was 2.6 ± 1.9 ng/min (mean values ± SEM).
Fig. 4.
Mean insulin release during perfusion of pancreas from 4 normal C57BL/KsJ-mice with 0.1 mM 3-isobutyl-1-methylxanthine added to the medium. 3-isobutyl-1-methylxanthine was added to the medium during the 30 min of pre-perfusion with 3 mM D-glucose and the subsequent 45 min of perfusion with 20 mM D-glucose. Glucose concentration was changed at time zero on the abscissa. The basal release 3 min before exposure to 20 mM glucose was 1.3 ± 0.3 ng/min, the peak response to 20 mM glucose was 220.6 ± 70.6 ng/min, and the release during min 20–25 was 10.3 ± 4.1 ng/min (mean values ± SEM).

Fig. 5.
Mean insulin secretion during repeated perfusion of pancreas from 2 normal C57BL/KsJ-mice with 20 mM D-glucose. The experiments were started by pre-perfusing the pancreas with 3 mM D-glucose for 30 min. At time zero on the abscissa the glucose concentration was then suddenly raised to 20 mM. Stimulation was interrupted during a rest period between min 20 and 30, when the perfusate contained 3 mM D-glucose. The glucose concentration was then again increased to 20 mM. The basal release 3 min before exposure to 20 mM glucose was 0.1 ± 0.1 ng/min, the first peak response to 20 mM glucose was 17.8 ± 5.6 ng/min, and the second peak response to 20 mM glucose was 11.3 ± 0.8 ng/min. The release during min 12–15 was 5.3 ± 0.9 ng/min and during min 43–45 5.1 ± 0.5 ng/min (mean values ± SEM).
(not shown), whether the glucose concentration was 30 or 20 mM (not shown), whether the medium contained 2.56 or 8 mM Ca\(^{2+}\) (Fig.3), and whether or not the medium was supplemented with 5 mM pyruvate, 5 mM glutamate and 5 mM fumarate (not shown).

Like physiological insulin secretion, both the initial transient spike and the second phase of constant insulin release were inhibited by omission of Ca\(^{2+}\) (Fig.2) and markedly enhanced by addition of 3-isobutyl-1-methylxanthine (Fig.4).

Insulin release during the constant phase was reversed by lowering the glucose concentration (Fig. 5). A second rise of glucose from 3 to 20 mM produced a secretory pattern very similar to the first response.

Fig. 6 summarizes how the basal secretory rate at 3 mM glucose was influenced by the various experimental factors. In absolute terms it was higher in rats than in mice, nearly identical in non-inbred mice and C57BL/KsJ-mice, enhanced by 3-isobutyl-1-methylxanthine, and very little, if at all, influenced by changing the Ca\(^{2+}\) concentration. Taking the size difference between rat and mouse pancreas into account, the basal insulin release per unit tissue was of the same order in the two species.

Discussion

The results show that both the rat and the mouse pancreas exhibit a biphasic release of insulin when perfused with a constant stimulatory concentration of glucose. The phenomenon of biphasic secretion is commonplace and has been demonstrated repeatedly with the rat pancreas (Grodsky et al. 1967; Curry et al. 1968; Bennett & Grodsky 1969) as well as with other endocrine systems (Iversen 1971; Brown & Feldberg 1936; Sherwood et al. 1968; Urquhart & Li 1968; Sachs & Haller 1968). However, the present experiments also show that the shape of the second secretory phase differs between species so closely related as the rat and the mouse. When tested in the same perfusion apparatus, the rat pancreas exhibited a second phase of slowly increasing secretory rate whereas in the mouse the second phase was approximately constant. Because more perfusion studies of the pancreas have been performed in rats, one is often tempted to believe that the pattern of insulin release observed in that animal is an inherent property of the mammalian β-cell in general. A previous study in the perfused mouse pancreas also tended to indicate that the secretory dynamics is very similar in rats and mice (Laube et al. 1973).

Therefore, my first interpretation of the present mouse data was that they are in some way artificial and distorted by the experimental procedure. However, despite considerable efforts to detect any reason for such distortion of the second phase in mice, none has been found.

Since the rat and the mouse pancreas were perfused at the same hydrodynamic pressure the difference in results is not due to pressure differences. Because the rat pancreas is bigger than that in the mouse, the constant pressure means that the total flow rate was greater in the rat. As indicated by direct oxygen measurements the difference on perfusion rate did not result in a lower arterial oxygen tension when perfusion of mice pancreas was carried out, since careful measures had been taken to prevent gas diffusion through the walls of tubing. Similarly, because of the possi-
bility that the mouse pancreas was inadequately supplied with nutrients the effect of adding pyruvate, fumarate and succinate to the perfusate was tested. This modification of the medium did not turn the constant second phase into one of increasing secretory rate. Neither could the results for mice be made more similar to those for rats by increasing the Ca\(^{2+}\) concentration in the perfusate.

Experiments were also performed to see whether the constant second phase in mice resembled physiological insulin release in three important respects: reversibility, potentiation by methylxanthine, and dependence on extracellular calcium ions. The results clearly show that the secretory rate during the second phase fell to basal level when the glucose concentration was lowered from 20 to 3 mM; both the sharp first phase and the constant second phase were reproduced when the glucose concentration was again introduced. In agreement with physiological insulin release, both the first and second phases in the perfused mouse pancreas were potentiated by 3-isobutyl-1-methylxanthine and inhibited by omission of Ca\(^{2+}\).

These results together support the conclusion that the constant second phase is not an artifact but a true characteristic of the perfused mouse pancreas. Although the cellular mechanisms responsible for the biphasic secretion are only tentatively understood, it has been suggested that the first phase represents a pool of insulin that is stored ready for immediate secretion near the \(\beta\)-cell plasma membrane. The second phase, on the other hand, may be more dependent on intracellular mechanisms for the transport of secretory granules up to the vicinity of membrane (Lacy et al. 1968; Orci et al. 1969; Devis et al. 1974; Malaisse et al. 1974). It does not seem inconceivable that various animal species differ in the detailed organization of the intracellular mechanisms for granule transport. It must be emphasized that a constant second phase is not entirely without precedence, since Curry et al. (1975) reported the second phase to be constant in the perfused pancreas of Syrian hamster and Gerhards & Rühl (1974) in Chinese hamster.

A second important result of the present study is that similar secretory dynamics, with a constant second phase, were observed in both non-inbred mice and inbred C57BL/\textit{KsJ}-mice. The C57BL/\textit{KsJ} genetic background is requisite for the mutated genes, \textit{db} or \textit{ob}, to produce diabetes with \(\beta\)-cell failure in mice (Hummel et al. 1972; Coleman & Hummel 1973). This disposition to a severe form of diabetes, as contrasted with the mild hyperglycaemia and \(\beta\)-cell hyperplasia induced by \textit{db} or \textit{ob} on other genetic backgrounds (Hummel et al. 1972), does not seem to be linked to the failure of the second phase to rise progressively as in rats.

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References


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