Glucagon and somatostatin secretion from the perfused splenic bulb of duck pancreas

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Abstract. Glucagon, somatostatin and insulin secretions were evaluated in a new type of perfusion preparation: the naturally A and D cell rich splenic bulb of duck pancreas. Stable basal levels were observed with 11 mM glucose, corresponding to normoglycaemia, and all secretions were stimulated by 1 mM 3-isobutyl-1-methylxanthine and by 10 mM arginine, demonstrating the technique's validity. In the absence of aminoacids in the perfusion medium, A cell blindness to glucose was corrected by physiological levels of insulin (2 ng/ml); insulinodependency of A cells, and unresponsiveness of D cells to glucose, probably not ruled by insulin, were observed. However, in the presence of aminoacids, glucagon was inhibited and somatostatin secretion stimulated by glucose (33 mM), independently of insulin (2 ng/ml). Aminoacids greatly influenced pancreatic hormone release.

Pancreatic A and D cell secretions are not fully understood, especially the modulation by insulin of the A cell's response to glucose: although insulin is now considered to play a key role in the regulation of glucagon secretion in response to glucose in vivo (Unger & Orci 1981; Laurent & Mialhe 1976) with some exceptions (Asplin et al. 1983), conflicting results have been reported in vitro (Ostenson 1979; Gorus et al. 1984). The response of D cells to glucose and its eventual modulation by insulin leads also to divergent results: glucagon stimulates somatostatin secretion in vitro in mammals (Hermansen 1980) and in the chicken (Honey et al. 1980), however no stimulation could be observed in the duck neither in vitro (Gross & Mialhe 1986) nor in vivo (Laurent et al. 1985). In the same way, there are discrepancies about the rôle of insulin in the response of D cells to glucose (Hermansen et al. 1980).

This can partly be explained by the limited number of secretion models available for studies of even partially segregated A and D from B cells. In mammals, all endocrine cell types are mixed in islets (Unger & Orci 1981), while in birds, A and D cells are mostly segregated from B cells in diffuse glandular cords, with separated B cell islets (Mialhe 1958; Laurent 1983). Moreover, these A and D cell cords are quantitatively more important than the B cell islets in the splenic round anterior end of the duck pancreas, which is called here splenic bulb. This morphological point is corroborated by hormone content measurements, and by physiological data in this species: subtotal pancreatectomy, when the splenic bulb is left, induces hyperglycaemia, while total pancreatectomy provokes hypoglycaemia (Mialhe 1958).

So, in order to provide a practical and dynamic method for the study of A and D cell secretion and their eventual regulation by insulin, we have perfused the splenic bulb of the duck, which is a natural relatively insulinodefficient part of the pancreas. The validity of this new preparation was checked by stimulation tests using 3-isobutyl-1-methyl-xanthine and arginine. The response of A and D cells to glucose, and their eventual modulation by insulin, was then studied. Moreover, since aminoacids influence pancreatic hormone release (Pagliara et al. 1974; Gerich et al. 1974), we used two perfusion media with and without physiological amounts of aminoacids.
Material and Methods

1. Animals and experimental procedures

Three months old adult male Peking ducks (2 to 2.5 kg), fed on a commercial fowl diet with vitamins, cereals and animal proteins (Grands Moulins de Paris, Nancy, France), were fasted overnight. They were tied on a board, and their abdomen was incised under local anaesthesia (xylocaine 1% Bellon); the animals remained calm and immobile under these conditions. The vessels to the gizzard, the spleen and the intestine were ligated. After preparation of ligatures 1, 2, 3 and 4 (Fig. 1), the terminal bulb of the splenic lobe of the pancreas was rapidly perfused in situ: the afferent catheter was cannulated, with a slow perfusion rate, into the pancreaticoduodenal artery at the level of the gizzard (ligature 1); the animals were then rapidly decapitated with the normal perfusion rate (2 ml/min), ligatures 2 and 3 tied and the efferent catheter carefully introduced at the gizzard’s level into the portal vein (ligature 4).

2. Perfusion media and experimental apparatus

The perfusate was a Krebs Ringer bicarbonate buffer (24.9 mM NaHCO₃, 119 mM NaCl, 4.78 mM KCl, 2.52 mM CaCl₂·2H₂O, 1.16 mM KH₂PO₄, 1.16 mM MgSO₄·7 H₂O), pH = 7.4 (Laurent 1983), with 0.5% dextran T 70 (Pharmacia), 0.2% bovine serum albumin (Sigma) and 11 mM glucose (D + glucose monohydrat, Roth). When mentioned in the experiments, the ten quantitatively most important aminoacids in duck plasma were added to the medium at their plasmatic concentrations i.e.: Thr 1.24 mM; Ala: 1.08; Ser: 0.66; Lys: 0.56; Leu: 0.49; Gly: 0.48; Val: 0.34; Ile: 0.29; Pro: 0.26 and Arg: 0.25 mM (Laurent et al. 1985). This medium was subsequently used to prepare control perfusate (11 mM glucose) and experimental perfusates with the following components: 1 mM 3-isobutyl-1-methyl-xanthine or IBMX (Sigma) + 11 mM glucose; 10 mM L-Arginine Monochlorid (Merck) + 11 mM glucose; 33 mM glucose; 33 mM glucose + 2 ng/ml insulin (MC porcine Actrapid Novo). A 95% O₂−5% CO₂ mixture was constantly gassed over the perfusate. The flow rate was maintained with a peristaltic pump (P3, Pharmacia), between 1.8 and 2 ml/min, which reproduced the pancreaticoduodenal artery pressure. The perfusate temperature was adjusted to 42°C, the duck body temperature, by passing through a warming cabinet. The splenic bulb was covered with a saline-soaked gauze and maintained at a moist 42°C by a heat lamp throughout the experi-

![Fig. 1.](image)

Perfusion technique of the splenic bulb in the duck pancreas (the dorsal lobe is not shown).
ment. The system was allowed a 30 min stabilization period before experiments were begun. Temperature and pH were checked at the end of each experiment, and no oedema was observed.

3. Assays

**Glucose.** Perfusate glucose was checked by the Hoffmann's method (1937) adapted to an autoanalyzer Technicon.

**Insulin.** Immunoreactive insulin (IRI) was determined by the method of Foltzer & Mialhe (1976) using beef insulin as a standard: the results are expressed as ng beef IRI equivalents. At the concentrations used, Laurent & Mialhe (1976) showed that beef, pork and duck insulin cross-react in a similar way with our antibody until 0.8 ngEq/ml, which covers the range of concentrations generally observed in our experiments; higher amounts of duck insulin would be about 2-fold underestimated, and the elevated porcine insulin, used in Figs. 5 and 7, leads to an overestimation. The sensitivity is 0.1 ng/ml and the intra- and inter-assay coefficients of variation are 7 and 10%, respectively.

**Glucagon.** Immunoreactive glucagon (IRG) was estimated by the method of Leclercq-Meyer et al. (1970), using porcine glucagon as a standard; hence the results are given as ng equivalents of porcine glucagon, which has been shown to compete like avian pancreatic extracts with $^{125}I$ porcine glucagon in our assay (Karmann, personal communication). The sensitivity was 0.1 ng/ml and the assay had intra- and inter-assay coefficients of variation of 4 and 10%, respectively.

**Somatostatin.** Immunoreactive somatostatin (IRS) was determined by the radioimmunoassay of Di Scala (1983), using synthetic S-14 as a standard, which competed like pancreatic effluents with our antibody (Gross & Mialhe 1986). The sensitivity of the assay was 10 pg/ml and intra- and inter-assay variation averaged 4.9 and 8.9%, respectively.

4. Calculations and statistical analysis

Hormonal output per min (ng/min) was determined by multiplying the concentration (ng/ml) by the flow rate (ml/min). Increments or decrements in hormone release during a stimulation were calculated by cumulating the variations in hormonal output during the corresponding 10 min period; basal stimulatory output was subtracted.

Student's paired t-test was used for determinations of statistical differences within a same experiment: the values were compared to the determination preceding the stimulus (−2 to 0 min). Student's unpaired t-test was used for comparison of cumulated variations in hormone output in the presence and in the absence of insulin. Results are expressed as mean values, with standard error of the mean (SEM).

**Results**

Basal secretion of insulin, glucagon and somatostatin was measured every 2 min with 11 mM glucose, i.e. normoglycaemia (200 mg/dl) in the duck. No significant difference could be detected when compared to the prestimulatory fraction in each experiment. Basal insulin output was about 1 ng/min throughout the experiments; glucagon basal release was increased from 1 to 5 ng/min in the presence of amino acids, while somatostatin basal output was more variable from one experiment to the other (3 to 5 ng/min).

1. **Effect of 3-isobutyl-1-methyl-xanthine (IBMX)**

An exposure of the splenic bulb to 1 mM IBMX resulted in an immediate increase in glucagon release ($P < 0.01$ from 6 to 14 min; $P < 0.05$ from 0 to 6 and from 14 to 18 min) and a delayed
Arginine I0 mM

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Insulin (IRI), glucagon (IRG) and somatostatin (IRS) secretions during splenic bulb perfusions with 10 mM arginine (and 11 mM glucose) for 10 min. • and ○ same symbols as in Fig. 2 (n = 6).

stimulation of insulin secretion (P < 0.01 from 6 to 8 min; P < 0.05 from 4 to 6 and from 8 to 16 min). An important and sustained stimulation of somatostatin release occurred from 2 to 18 min (P < 0.05) (Fig. 2).

2. Effect of arginine

When the splenic bulbs were exposed to 10 mM arginine, a very important stimulation of glucagon release occurred: a 16-fold magnification of the secretion rate was observed 4 min after the exposure (P < 0.05 from 1 to 4 and from 10 to 14 min, P < 0.01 from 4 to 10 min); insulin (P < 0.05 from 0 to 14 min), and somatostatin (P < 0.05 from 3 to 10 min) secretions were also stimulated, although at a lesser extent (Fig. 3).

3. Effect of glucose

Physiological amounts of glucose (33 mM, i.e. a 3-fold increase compared to basal levels) induced a slight, but significant increase in insulin secretion (P < 0.05 from 0 to 2 and from 8 to 12 min; P < 0.01 from 2 to 8 min), but no significant variation in glucagon release was observed with even a non-significant rise from 0 to 2 min. A very small, short and delayed rise in somatostatin release (P < 0.05) occurred from 4 to 8 min (Fig. 4). The mean variations in glucagon and somatostatin release, cumulated during the 10 min stimulation period were respectively −1.09 ± 1.37 ngEq and +33.98 ± 12.41 ng.
4. Effect of glucose and insulin

The amounts of insulin used (2 ng/ml) corresponded to a doubling of the insulin release observed after a glucose injection in vivo (Laurent & Mialhe 1976).

When the splenic bulbs were exposed to 33 mM glucose in the presence of physiological amounts of insulin (2 ng/ml) an immediate decrease in glucagon secretion occurred ($P < 0.05$ from 0 to 10 min), while no variation in somatostatin release could be detected (Fig. 5). The mean decrement in glucagon release, cumulated during the 10 min stimulation period, was $-11.34 \pm 3.35$ ngEq, which was significantly different ($P < 0.01$) from the variation observed without insulin. However, no significant difference could be detected between the somatostatin variations observed during the glucose stimulation with $(+4.26 \pm 8.29$ ng) and without insulin.
5. Effect of glucose in the presence of amino acids (Fig. 6)

When amino acids were added to the perfusion medium in a physiological concentration (see Material and Methods), 33 mM glucose elicited a small but significant rise in insulin secretion ($P < 0.05$ at 2 min; $P < 0.01$ from 2 to 18 min), an important decrease in glucagon release ($P < 0.01$ from 2 to 10 min) and a large stimulation of somatostatin secretion ($P < 0.01$ from 2 to 12 min). The mean decrements in glucagon and increments in somatostatin, cumulated during the 10 min stimulation period, were respectively $-31.37 \pm 6.19$ ngEq and $+37.59 \pm 6.30$ ng.

6. Effect of glucose and insulin in the presence of amino acids

In this case, when the splenic bulbs where exposed to 33 mM glucose in the presence of physiological amounts of insulin (2 ng/ml), a decrease in glucagon secretion could be observed from 2 to 10 min ($P < 0.01$), and an important stimulation of somatostatin release ($P < 0.01$ from 2 to 12 min) occurred, as in the absence of insulin in the perfusion medium (Fig. 7). No significant difference could be detected, during the 10 min stimulation period, neither between the cumulated glucagon decrements observed with ($-31.16 \pm 5.18$ ngEq) and without insulin, nor between the cumulated somatostatin increments calculated in the presence ($+34.86 \pm 5.25$ ng) and in the absence of insulin.

Discussion

The main purpose of this work was to find a practical model for studying the dynamics of A and D cell secretion. In view of the natural relative insulinodeficiency of the splenic bulb in ducks, we chose to perfuse this part of the pancreas. The validity of this new type of preparation is described here, and the responsiveness of A and D cells to glucose, as well as their modulation by insulin and amino acids, are discussed.

Validity of the perfusion technique

Basal secretions were stable. Moreover, arginine as well as IBMX, stimulated all the secretions. These data provide good arguments for the validity of this new perfusion technique.

Basal secretions

When compared to the perfused chicken pancreas (Honey et al. 1980), where the splenic part is excluded, basal secretion of insulin was 2-fold lower, but glucagon and somatostatin outputs.

![Figure 6](image)

**Fig. 6.** Effect of glucose in the presence of amino acids (AA).

![Figure 7](image)

**Fig. 7.** Effect of a simultaneous exposure of splenic bulbs to 33 mM glucose and 2 ng/ml insulin, in the presence of amino acids (AA), for 10 min, on glucose level, insulin (IRI), glucagon (IRG) and somatostatin (IRS) release. ** and o: same symbols as in Fig. 2 (n = 7).
were respectively 2- to 10-fold (depending on the presence or not of aminoacids in the medium) and 5- to 7-fold higher, in the perfused splenic bulb of the duck. These elevated somatostatin and glucagon and relative low insulin release correlated well with morphological and physiological data (Laurent 1983), and with a 4-fold higher glucagon and somatostatin versus insulin content (Laurent, unpublished data) of this part of the duck pancreas.

**Effect of 3-isobutyl-1-methyl-xanthine or IBMX**

IBMX induced a rise in insulin, glucagon and somatostatin secretions. A similar stimulation of pancreatic hormones was observed by Jarousse & Rosselin (1975) in the rat and by Foltzer et al. (1982) in the chicken with theophylline. Since methylxanthines are known to potentiate cAMP by phosphodiesterase inhibition, our data further substantiate the stimulating effect of cAMP on insulin, somatostatin (Hermansen 1980) and, especially, glucagon release. However, the delayed insulin stimulation could be secondary to the increase in glucagon, the insulinogenic properties of which are well known (Samols & Harrison 1976).

**Effect of arginine**

Arginine stimulated insulin and somatostatin secretions, the effect on glucagon being even more marked. A similar stimulation of insulin and glucagon secretions, observed in vivo in the duck (Laurent & Mialhe 1978), has also been reported for somatostatin in the perfused pancreas of mammals (Hermansen 1980) and chicken (Honey et al. 1980). Our data suggest the existence of a direct effect of arginine on A, B and D cells in the duck, as it is the case in mammals.

**Effect of glucose. Rôle of insulin**

The physiological amount of glucose used in this study (33 mM glucose) has been shown to be the most efficient stimulus in organ culture of chick endocrine pancreas (Foltzer et al. 1982). Pig insulin, which differs from duck insulin by 6 aminoacids, has been used in our experiments. Though chicken insulin exhibits higher affinities and activities than pig insulin in mammalian tissues (Simon et al. 1977), little is known about the potency of pig compared to avian insulin in the duck. However, 2 ng/ml pig insulin (this concentration corresponds to a doubling of the endogenous insulin release observed in vivo in the duck after a glucose injection) induce A cell inhibition in the absence of endogenous B cell stimulation in diabetic ducks (Laurent & Mialhe 1976).

a) **Insulin output**

Insulin secretion from the splenic bulb was weakly, but significantly, stimulated by glucose with or without aminoacids in the perfusion medium. This weak response can be explained by the relative insulinodeficiency of this part of the pancreas, but also by the relative insensitivity of the avian B cells to glucose in vitro (Honey et al. 1980; Gross & Mialhe 1986). When comparing our results to those of Gross & Mialhe (1986), the in vitro threshold of sensitivity of duck B cells to glucose is situated between 22 and 33 mM glucose.

b) **Glucagon response to glucose in perfused splenic bulbs. Rôle of insulin.**

A cells were insensitive to 33 mM glucose, but physiological amounts of insulin (2 ng/ml) restored a normal response to glucose when aminoacids were absent from the perfusion medium. However, in their presence, inhibition of A cells by glucose could be observed, and the adjunction of insulin did not modify this response. So, in our model, the response of A cells to glucose is not insulinodependent in the presence of aminoacids.

It is interesting to compare these data with the results obtained in the duck in vivo: glucose stimulates insulin secretion and inhibits glucagon release in normal animals, while no variation of both insulin and glucagon, or even increased glucagon levels, are observed during hyperglycaemia in two days subtotally pancreatectomized animals, in which only the splenic bulb is left (Laurent & Mialhe 1976): these animals are diabetic, with basal hyperglycaemia and decreased glucose tolerance. However, physiological insulin therapy in these diabetic animals restores a normal response of A cells to glucose (Laurent & Mialhe 1976).

The rôle of insulin in the response of A cells to glucose is controverted in vivo as well as in vitro.

The existence of a rôle of insulin in the response of A cells to glucose has been established in some conditions. Insulinodependency of A cells has been reported in vivo in diabetic mammals (Assan et al. 1981; Unger & Orci 1981) and ducks (Laurent & Mialhe 1976). In vitro, a suppressive
effect of insulin on the response of A cells to glucose has been observed in guinea-pig islets (Östenson 1979) and in the perfused rat pancreas, even if it is indirect (Leclercq-Meyer et al. 1983).

However, in other conditions, glucagon suppression by glucose is insulin-independent, as observed in vitro in our perfused splenic bulbs in the presence of amino acids, as well as in perfused ventral and dorsal lobes of duck pancreas (Gross & Mialhe 1986: amino acids were present in the medium) but also with FACS isolated A cells (Gorus et al. 1984), in the perfused rat pancreas (Gerich et al. 1974; Pagliara et al. 1974) and in vivo in man by Asplin et al. (1983).

So, in the perfused splenic bulb of duck pancreas, glucose could be viewed as an inhibitor of A cell secretion; if no stimulus is present, glucose inhibition of release is not noticeable and is only observed when a stimulus (i.e. amino acids) is present.

c) Somatostatin response to glucose. Role of insulin

Somatostatin response to glucose was very weak (in the absence of insulin), or nonexistent (in its presence) when amino acids were discarded from the perfusion medium. Conversely, in the presence of amino acids, somatostatin secretion was strongly stimulated by glucose in the presence as well as in the absence of insulin. The effect of glucose on somatostatin output seems to be insulin-independent and aminoacids greatly influence D cell release.

In normal, as well as in diabetic, subtotally depancreatized ducks (the splenic bulb is left), glucose does not elicit any response of D cells. Similar observations were made in low dose glucose perfused dorsal and ventral lobes of duck pancreas (Gross & Mialhe 1986) as well as in the perfused pancreas of the diabetic dog (Hermansen 1980). A stimulation of somatostatin secretion by glucose, as observed in our system with physiological amounts of aminoacids in the perfusion medium, has been described in vitro in normal mammals (Hermansen 1980) as well as in the perfused chicken pancreas (Honey et al. 1980), but only during hypoglycaemia. Similarly, insulin has no demonstrable effect on somatostatin secretion of mammalian pancreata (Hermansen 1980); however, an inhibitory effect has been described during hyperglycaemia in the perfused rat pancreas (Gerber et al. 1981) and during diabetes in vivo in the dog (Hermansen et al. 1980).

Conclusion

Our studies on perfused duck pancreatic splenic bulbs show that:

1. The secretion of glucagon is not insulin-dependent if aminoacids are present.
2. The secretion of somatostatin is not insulin-dependent, but is stimulated by glucose only if aminoacids are present.
3. Aminoacids greatly influence A and D cell secretion.

Acknowledgments

We wish to thank Miss Horrenberger, Mrs Roth and Sommermeyer for valuable technical assistance and Miss Schwartz for typing the manuscript.

This work was supported by the CNRS (ERA 188), the INSERM (contrat No. 75105104) and the Fondation pour la Recherche Médicale.

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Received November 22th, 1985.

Accepted April 24th, 1986.

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