A Technique for the Perfusion of the Isolated Rabbit Pancreas

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Abstract—The present paper describes a technique for the vascular perfusion of the isolated rabbit pancreas, evidence for the viability of the perfused gland and the responses to stimulation by secretin (Sc) and acetylcholine (ACh). The isolated rabbit pancreas was perfused at a constant pressure through the superior mesenteric artery with Krebs-Henseleit solution. The perfused gland, kept in a viable state over the perfusion period of 7 hr, secreted pancreatic juice spontaneously; the juice flow increased and the amylase output decreased gradually for approx. 2 hr after the beginning of perfusion, following the flow and output relatively constantly for several hours. The single infusion of Sc caused a prolonged flow of the pancreatic juice with an increase in the bicarbonate concentration and a decrease in the chloride concentration, though Sc was present in the perfusate for only a short time. The single infusion of ACh was not effective on the juice flow, but immediately increased the amylase output dose-dependently. These results indicate that the saline-perfused preparation of the rabbit pancreas has wide applications in experimental studies on the exocrine pancreas.

It is well known that the exocrine pancreas, which has an anatomy and behaviour differing considerably in a wide variety of animal species, is regulated by various factors such as the nervous system, hormones and blood flow. Because analysis in the intact gland is complicated by the influence of variable control mechanisms, a technique was recently developed for vascular perfusion of the isolated pancreas in different species.

In the dog, Hermon-Taylor and Chir (1) developed a technique for vascular perfusion of the isolated pancreas with heparinized blood, whereas in situ methods for vascular perfusion of dog pancreas have been presented by Goldstein (2) and Nardi et al. (3). In the cat, Case et al. (4) described in detail a technique for vascular perfusion of the isolated pancreas with a saline solution containing secretin. In the rat, Khayanbashi and Lyman (5) applied the perfusion apparatus, which was developed for the study of endocrine pancreas by Long (6), to investigate amylase release from the isolated gland perfused with plasma. On the other hand, Kanno (7) developed an alternate technique for perfusing the isolated pancreas with modified Krebs-Henseleit solutions, though Ueda (8) later described a similar technique in a short communication. In the rabbit, a technique for perfusion of the isolated pancreas has not yet been reported, whereas Rothman (9) and Rothman and Brooks (10) studied the mechanism of electrolyte secretion using an isolated pancreas immersed in an organ bath filled with saline solution.

The present experiments show the technique for perfusion of rabbit pancreas, the evidence for viability of the perfused gland and the responses to secretagogues. Some of the present results have been published in abstracts (11, 12).

Materials and Methods

Vascular supply of rabbit pancreas: In the rabbit, a large part of the pancreas is located within the meso-duodenum of the duodenal loop (Fig. 1). The stem arteries in the pancreas start from the superior mesenteric and celiac arteries. From the superior mesenteric arterial system, the inferior pancreatico-
duodenal branch supplies a larger part of the gland, anastomosing with the branch of the celiac artery. All the stem veins in the pancreas run into the portal vein. Thus, perfusion fluid is infused into the gland's arterial supply via a cannula in the superior mesenteric artery and is drained to the portal vein.

Isolation and perfusion of the pancreas: Japanese white rabbits of either sex, weighing 2.5–3.0 kg, were fasted but allowed water for 24 hr before the experiments. Under Nembutal anaesthesia (sodium pentobarbital, 40 mg/kg intravenous injection), the abdomen was opened, and the attachments of other intestinal segments to the duodenal loop were cut. Leaving the duodenal loop, the other intestine was removed after the superior mesenteric artery was ligated and cut distal to the inferior pancreaticoduodenal artery, which supplies part of the duodenum and pancreas. A small loop of the rectum attached to the omental surface was left in order to prevent damage to the pancreas. The perfusion fluid was infused into the superior mesenteric artery after cannulation with a polyethylene tube (1.6 mm o.d.) and is drained from the cannula (3 mm o.d.) of the portal vein. The animal was then killed by cutting the carotid arteries, and the preparation that consists of the pancreas, duodenum and attached rectum was entirely isolated by cutting it off at the root of the duodenal loop which was previously nipped with a clip (stainless steel). The isolated preparation was mounted on a cork frame by pins through various portions of the duodenum. To prevent edema of the pancreas, the duodenum was opened in some places and a part of the perfusate was allowed to leak out. The cork frame was then placed in a chamber after cannulation in the main pancreatic duct with a polyethylene tube (1 mm o.d.). Both the pancreatic duct cannula and portal vein cannula passed through a hole in the chamber wall. Approximately 45 min was required to mount the preparation in the chamber after the abdomen was opened.

A diagram of the perfusion apparatus and the preparation set in the chamber is shown in Fig. 2. Perfusion fluid, which was led from a reservoir through a heat-exchange coil in

\[\text{Fig. 1. Schematic representation of the rabbit pancreas and its vascular supply. AA, abdominal aorta; CA, celiac artery; D, duodenum; IPA, inferior pancreaticoduodenal artery; LA, lienal artery; P, pancreas; PD, pancreatic duct; PV, portal vein; R, rectum; SMA, superior mesenteric artery; SPA, superior pancreaticoduodenal artery.}\]
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a water-bath kept at 37°C, was supplied to the preparation at a constant pressure of 25 mm Hg provided by a large reservoir of mixed gas (O₂ 95%, CO₂ 5%). The perfusate from the portal vein and the leakage solution from the duodenum were separately collected in vessels for the measurement of each flow rate; in ten experiments, the mean flow rate of the former was 3.1 ±0.1 S.E. ml/min, and the latter was 1.9 ±0.1 ml/min. Acetylcholine chloride (ACh) and secretin (Sc, Boots), which were freshly prepared in each experiment, were infused through a side arm of the cannula into the superior mesenteric artery. Single infusion was carried out with 5 ml for 1 min. The dose of Sc was expressed in Crick, Harper and Raper (13) units per milliliter solution. The perfusion was usually carried out with Krebs-Henseleit solution of the following composition (mM): NaCl, 118.6; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; NaHCO₃, 24.9; MgCl₂, 1.2; and glucose, 5.5. The solutions were equilibrated with mixed gas (O₂ 95%, CO₂ 5%) and had a pH of approx. 7.4.

Assay of pancreatic juice: The pancreatic juice was collected over 10 min periods in small weighed tubes and output determined gravimetrically. Amylase was assayed by the method of Smith and Roe (14) with the following modification: Soluble starch (Wako Pure Chemicals, Japan) was used instead of Litner's starch. Bicarbonate was estimated by a Natelson microgasometer (Kayagaki, Japan) with 10 µl samples which were collected under paraffin oil. Chloride was determined titrimetrically by the method of Schales and Schales (15). Sodium and potassium were measured with a flame photometer (Hitachi 208, Japan).

Results

Spontaneous secretion: The spontaneous secretion from the isolated rabbit pancreas perfused with Krebs-Henseleit solution, varied in different preparations, was obtained for up to at least 7 hr without external stimulation (Fig. 3). The rate of juice flow
increased with time during the first 2 hr, but kept relatively constant for the following 5 hr. The amylase output decreased gradually during the first 2 hr, but kept constant during the following 5 hr. When the temperature of water in the water-bath was lowered from 37°C to approx. 4°C in three preparations, the juice flow was rapidly decreased and was almost abolished at the prolonged period of perfusion. This effect was reversible with a rise in the temperature.

In order to compare with the perfused glands, the spontaneous secretion of the intact glands was observed in rabbits anaesthetized with Nembutal. The juice flow in the intact gland increased slightly with time, whereas the amylase output was relatively constant for 7 hr after the duct cannulation (Fig. 3). Table 1 shows the mean values for the juice flow, the amylase output and the electrolyte concentration of secretory sodium, potassium, bicarbonate and chloride in the perfused and intact glands. The mean juice flow in the perfused gland was greater than...

**Fig. 3.** Time courses of the spontaneous secretion of pancreatic juice and amylase output. The solid line represents the mean±S.E. of these values in 5 perfused glands, and the broken line represents the mean±S.E. of these values in 4 intact glands.

**Table 1.** Spontaneous secretion in perfused and intact pancreas

|                      | In perfused glands | In intact glands |
|----------------------|-------------------|-----------------|
| Juice flow           | 5.65±0.54         | 4.28±0.42       |
| (mg/min)             | (10)              | (8)             |
| Amylase output       | 5.84±0.70         | 7.20±0.88       |
| (u/min)              | (10)              | (8)             |
| Na⁺                  | 146.90±1.43       | 144.13±1.70     |
| (m-equiv/min)        | (10)              | (8)             |
| K⁺                   | 6.81±0.14**       | 3.26±0.19       |
| (m-equiv/min)        | (10)              | (8)             |
| HCO₃⁻                 | 71.25±2.76*       | 54.13±5.16      |
| (m-equiv/min)        | (8)               | (8)             |
| Cl⁻                  | 89.50±3.06        | 99.13±6.65      |
| (m-equiv/min)        | (8)               | (8)             |
| HCO₃⁻+Cl⁻             | 160.75±1.57*     | 153.25±1.82     |
| (m-equiv/min)        | (8)               | (8)             |

Pancreatic juice was collected for 20 min following the first 2 hr after perfusion (in perfused glands) or duct cannulation (in intact glands). Values for juice flow (in mg/min), for amylase output (in u/min) and for ion concentrations (in m-equiv/min) are means±S.E., and the number of experiments is shown in parentheses. Statistical significance: *P<0.05, **P<0.001, compared with each value in intact glands.
that in the intact gland, whereas the mean amylase output was slightly less than that in the intact glands. In all experiments, the concentrations of sodium and potassium were independent of the rate of the juice flow and were sustained throughout the experiment. The sodium concentration in the perfused gland was similar to that in the intact gland, whereas the potassium concentration in the perfused gland was approx. 2-fold that in the intact gland. The concentrations of the major anions, bicarbonate and chloride, varied with the rate of the juice flow. The mean concentration of bicarbonate in the perfused glands was significantly greater than that in the intact glands, whereas the mean chloride concentration in the perfused glands was less. The sum of the bicarbonate and chloride in the perfused glands significantly exceeded the sum of the two anions in the intact glands.

Response to secretin: In the spontaneous secretion of the perfused glands, it was found that the secretory rate and amylase output did not remain constant for the first 2 hr period after the beginning of perfusion. Therefore, the response of the resting pancreas to single infusions of secretin (Sc) was investigated in the following 5 hr period. In six preparations, doses of Sc from 5 to 1000 μu/ml were infused into the arterial cannula. When the concentration of Sc was increased up to 10 μu/ml, the rate of the juice flow was enhanced for a long time; the juice flow increased rapidly to a maximum at the beginning of the response and then gradually declined (Fig. 4). The bicarbonate concentration increased as the juice flow rate increased, while the chloride concentration decreased as the juice flow rate increased. The concentrations of sodium and potassium remained nearly constant during the experimental period.

Response to acetylcholine: In five preparations, the response of the perfused gland to single infusions of acetylcholine (ACh) was investigated in the following 5 hr period after the first 2 hr of the perfusion. When the concentration of ACh was increased up to 5×10^{-9} M, the output of amylase in the pancreatic juice was enhanced immediately.

![Fig. 4](image_url) Time courses of the pancreatic juice flow and the concentration of secretory bicarbonate and chloride in the response of the perfused pancreas to single infusions of secretin. At the point marked Sc, the gland was stimulated by secretin for one min.
Within the effective range, this effect increased with increasing concentrations of ACh (Fig. 5). The single infusion of ACh was not effective on the rate of juice flow, though it was frequently observed that high concentration of ACh inhibited the juice flow during the early period of infusion.

Discussion
The pancreas of the rabbit, whether isolated or in the intact anaesthetized preparation, spontaneously secreted ample pancreatic juice. This property is convenient for the simultaneous determination of several constituents in the pancreatic juice. Hermon-Taylor and Chir (1) have made a similar observation in the perfused dog pancreas, whereas Case et al. (4) have reported that the pancreas of a cat did not secrete unless Sc was administered. In order for the perfused pancreas to be experimentally useful, it is important for the perfused gland to behave similarly to the intact gland. As to the spontaneous secretion from the rabbit pancreas, Rothman (9) and Rothman and Brooks (10) showed that the isolated gland, immersed in a saline-filled bath, secretes at a near-maximal rate without external stimulation and produces 5-8 times more pancreatic fluid than in vivo. In the present experiments, the spontaneous secretion was very similar in the perfused and intact preparations, though in the perfused gland, the juice flow was slightly greater and the amylase output slightly less than that in the intact gland. The spontaneous secretion was strongly inhibited.

Fig. 5. Amylase output in the response of the perfused gland to successive infusion of various doses of acetylcholine at regular intervals of 40 min. The arrows indicate the periods at which acetylcholine was given.
when the perfusion fluid of low temperature was supplied in the isolated gland. However, this effect was reversible with a rise in temperature. These results suggest that the spontaneous secretion from the perfused pancreas is due to the active transport of electrolytes across the membrane of the secretory cells.

The concentrations of the secretory sodium and potassium, were independent of the rate of juice flow and were higher than those in the perfusate. Similar observations have been reported by Rothman and Brooks (10) in isolated rabbit pancreas and by Case et al. (4) in isolated perfused cat pancreas. Part of the excess of both cations has been assumed to be due to the glucose and ions such as calcium, magnesium and phosphate present in the perfusate (4), to which the pancreas has been shown to be relatively impermeable (16). In the present experiments the concentration of the secretory bicarbonate was approx. 2.8-fold more than that in the perfusate. Furthermore, an inverse relationship of the bicarbonate and chloride concentrations was observed with the increase of juice flow when the perfused pancreas was stimulated by Sc (Fig. 4). These observations suggest the viability of the isolated perfused pancreas.

Rothman and Brooks (10) have reported the response of the isolated rabbit pancreas, immersed in a saline-filled bath, to the stimulation of Sc and have observed that in 10 of 12 experiments, the addition of Sc (60 u/kg bathing solution) produced an increased secretory rate, of which the percentage change was small in many preparations. Furthermore, Ridderstap (17) investigated the response of the isolated rabbit pancreas to increasing doses (60-480 u/I bathing solution) of Sc and showed that a maximal average flow stimulation of 35% occurred after addition of 480 u/l of bathing solution. In the present experiments, the pancreatic juice was enhanced when the concentration of Sc was increased up to 10 mu/ml. The percentage in the juice flow was approx. 200–300% after a single infusion of 100 mu/ml of Sc (Fig. 4). These results have shown that the perfused pancreas kept high sensitivity to Sc and had a sensitivity similar to the intact gland.

ACh is a physiological secretagogue which accelerates the release of digestive enzymes from the pancreatic acinar cells. Kanno and Habara (18) have recently reported that the continuous stimulation of the isolated perfused rat pancreas with ACh induced flat increases in secretory responses; doses of ACh over the range 5×10^{-8} M – 10^{-6} M increased both amylase output and the rate of the juice flow. In the present experiment, the single infusion of ACh enhanced apparently the amylase output with dose-dependence within the effective range (5×10^{-9} M – 10^{-7} M), whereas ACh was ineffective on the rate of juice flow. These results suggest that the pancreatic acinar cell of the isolated perfused gland of rabbit would be very sensitive to ACh.

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