Perfusion System for Studying Peptide Secretion from Endocrine Cells with High Time Resolution

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ABSTRACT

To study the secretion from endocrine cells in culture, we have developed a cell column perfusion system with a time resolution of 4 s. The core of the system is a perfusion chamber with a cell-supporting matrix of monosized polystyrene beads. The particles are solid and can withstand a high pressure gradient without deformation. The minimum amount of cell material required to obtain detectable levels of secretory products is a function of the assay sensitivity, perfusion flow, fraction volume and time resolution. The volume of the perfusion chamber is therefore adjustable to satisfy varying demands of minimum cell number. The general flow characteristics of the system were characterized using radiolabeled substances of various molecular sizes. Using the system in secretory studies of rat pituitary tumor (GH_4C_1) cells, we have identified differences in secretion profiles that may be related to the kinetics of the different transmembrane and intracellular mechanisms involved.

INTRODUCTION

Kinetic studies are important to understand the regulation of hormone secretion. Many of the techniques currently used for such studies use tissue explants or primary cell cultures, both of which have disadvantages. Tissue explants suffer from diffusion barriers that lead to progressive anoxia and ultimately to necrosis of the tissue. The progressive tissue necrosis and the leakage of hormones from intracellular stores result in unreliable values for dose-response and time-course studies of hormone secretion. Cell cultures overcome the diffusion problem. However, they represent a static culture system that allows secretory and metabolic products with potential feedback effects to accumulate in the culture medium. In addition, cell cultures do not facilitate precise monitoring of transient changes in hormone secretion.

For these reasons, Lowry and McMartin (18) established the technique of cell column perfusion that was used in their studies of the control of steroid hormone secretion. The continuous flow of culture medium through the cell column ensures an efficient exchange of nutrients, secretagogues and secretory products between cells and medium and resembles the *in vivo* situation in most endocrine glands. The perfusion system has proved its usefulness in several studies of the regulation of pituitary hormone secretion (1,4,5,11,15–17,23,27,34).

Several new techniques that allow the study of exocytosis in the subsecond time-scale have been developed. These

techniques include membrane capacitance measurements (21), electrochemical detection of exocytosis (7,8,20) and optical detection of exocytosis (22). For the comparison and combination of some of these techniques, see the review by Angleson and Betz (2).

Using the patch clamp techniques (12), electrophysiological recordings from single cells in culture are routine today. It is also possible to measure the activity of the enzymes adenylyl cyclase and phospholipase C at intervals of a few seconds (4,5,19,25).

In most perfusion systems, the fractions have usually been collected at 0.5–30-min intervals, making it difficult or even impossible to study stimulus-secretion coupling by comparing the time-course of secretion and activation of cellular signaling systems. To describe more precisely such relationships, we have developed an optimized perfusion system with high time resolution. Here, we describe the system that allows measurement of prolactin (PRL) secretion from rat pituitary cells at 4-s intervals.

MATERIALS AND METHODS

Chemicals

Thyrotropin-releasing hormone (TRH) was obtained from Hoffman-La Roche (Basel, Switzerland) and human immunoglobulin G (IgG) from Kabi AB (Stockholm, Sweden). Ham's F-10 medium, sterile fetal calf serum (FCS), sterile horse serum and amphotericin B were purchased from Flow Laboratories (Maclean, VA, USA) and lactalbumin

min hydrolysate, penicillin-streptomycin and anti-pleuro pneumonia-like organisms (anti-PPLO) agent Tylocin from Life Technologies (Glasgow, UK). HEPES and ^{125}I were obtained from Sigma (St. Louis, MO, USA) and Iodogen from Pierce Eurochemie BV (Rotterdam, The Netherlands).

Cell Culture

The initial establishment of the GH

lines of rat pituitary cells from a transplantable rat pituitary tumor (MtT/W5) (28) was performed by Tashjian et al. (29,30). These cell strains spontaneously synthesize and secrete PRL and GH into the culture medium (14). Here, we used GH_4C_1 cells that produce PRL and only negligible amounts of GH. The cells were routinely cultured in a humidified atmosphere of 5% CO_2 and 95% air in complete Ham's F-10 medium containing 7.5% (v/v) horse serum and

2.5% (v/v) FCS using 150-cm² tissue culture flasks (Corning, Corning, NY, USA). Penicillin (50 U/mL), streptomycin (50 mg/mL) and Tylocin (60 mg/mL) were added to the culture medium and did not influence cell growth or hormone production. The culture medium was changed five days after subculture, and the cells were harvested by trypsinase [0.25% (w/v) for 30 s at 37°C] in the late logarithmic phase of growth. The trypsinase treatment did not reduce receptor binding compared with cells harvested by the gentle use of a scraping rubber policeman (data not shown). The cells were then resuspended in serum-free F-10 medium containing lactalbumin hydrolysate (5 mg/mL) and 20 nM HEPES, pH 7.4, supplemented with CaCl_2 [final Ca^{2+} concentration 1.2 mM] and immediately used for a perfusion experiment. This medium was designated F-10+.

Perfusion Chamber

The central part of the cell column perfusion system is the perfusion chamber, presented schematically in Figure 1. The main component of the chamber is a Perspex® tube with an inner diameter of 5 mm. The upstream end of the chamber is fitted with an adjustable plunger made of Delrin®. The 0.8-mm inlet to the chamber passes through the plunger, which has an inverted cone-shaped nozzle (20° angle) to secure a nearly homogeneous, axial flow through the chamber. At the downstream end, the chamber is fitted with a similar cone-shaped outlet. To avoid loss of matrix material and cells, a stainless-steel grid with a mesh size of 75 μm sections the narrow part of the output cone. The volume of the chamber can be varied from 0.2 cm³ to 1.5 cm³.

To attach the afferent and efferent silicone tubes (1/32 inch inner diameter; Cole Palmer, Vernon Hills, IL, USA), the plunger and the output end of the chamber are fitted with short stainless-steel pipes with an inner diameter of 0.8 mm.

Perfusion System

The cell column perfusion system is an improved version of previously described perfusion systems (3,11,16,

Figure 1. Technical drawing of perfusion chamber. (a) Input tube; (b) coned plunger; (c) perfusion chamber; (d) output tube; (e) perfusion chamber cylinder wall; (f) rubber o-ring; (g) perfusion chamber output cone; (h) stainless-steel grid; and (i) perfusion chamber output wall.

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18,27). Figure 2 shows a schematic representation of the perfusion set-up. The chamber (Figure 2a) is connected to a four-channel peristaltic pump (Ismatec, Switzerland) (Figure 2, c and d) through a set of pneumatically controlled three-way valves (model 5302; Rheodyne, Rohnert Park, CA, USA) (Figure 2e), which make it possible to switch rapidly between reservoirs containing different test solutions. Each reservoir is linked to the pump through an external flow loop during the periods when it is not used for perfusion. Simultaneous switching of the appro-

priate valves redirects the flow through the chamber when perfusion is required. This arrangement minimizes transients in the flow rate during the rapid changes of test solutions. The chamber is placed vertically in a water bath (Figure 2g) at 37°C, and the cell column is perfused in a downward direction. The medium reservoirs and the inlet tubing are also kept in the water bath during an experiment. The outlet tubing is connected to one of the channels of the peristaltic pump and facilitates flow through the cell column. The outlet fractions are collected at inter-

vals that vary with fraction volume and flow speed by using a fraction collector (ISCO Retriever II™; ISCO, Lincoln, NE, USA) (Figure 2h).

The pneumatic valve system and the fraction collector are controlled using a microcomputer and a custom-built 24-V power unit controlling the pneumatic valves via electromagnetic valves (Mecman, Sweden). An overflow chamber (Figure 2f) is connected to the perfusion chamber through a T-connector at the input tube close to the inlet to the perfusion chamber. The liquid level in the overflow chamber monitors the pressure within the perfusion chamber and prevents pressure build-up or vacuum in the perfusion chamber caused by a minor unbalance between input and output flow rates.

Typically, the perfusate is collected in 100-μL fractions (two drops). The variation in flow rate (time intervals between each drop) measured by a photo-cell connected to the microcomputer is normally less than 0.2% at a flow rate of 1.5 mL/min and a chamber volume of 0.7 mL.

Cell-Supporting Matrix

Before an experiment, the perfusion chamber plunger was removed, and approximately 0.1 cm³ matrix was layered at the bottom of the perfusion chamber on top of the output grid. The cells were gently mixed with approximately 0.6 cm³ cell-supporting matrix using a blunted pipet. The suspension was gently applied on top of the matrix layer at the bottom of the chamber. The matrix sedimented while standard medium was added on top of the cell-containing matrix. The plunger was then carefully lowered to the top of the column, eliminating the dead volume of the chamber. The afferent and efferent tubing of the perfusion chamber were then connected to the perfusion pump. The flow rate was gradually increased to the required level.

Before starting an experiment, the cell column was perfused for 15 min with medium to remove prolactin secreted during the packing of the cell column or leaking from damaged cells. The matrix used was either 70-mesh Bio-Gel® P6-DG (Bio-Rad Laboratories, Hercules, CA, USA) or solid, hy-

Figure 2. Perfusion system setup. (a) Perfusion chamber; (b) perfusion media/test solutions; (c) afferent peristaltic pumps; (d) efferent peristaltic pump; (e) three-way pneumatic valves; (f) overflow chamber; (g) water bath; and (h) fraction collector.

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drophobic, 80- or 73- μm monosized, cross-linked polystyrene spheres (Dynospheres® experimental type R-77 and EXP-SS-78.3, Dyno Specialty Polymers; Dyno ASA, Lillestrøm, Norway) with a variation in diameter of less than 5% (32). Before use, the monosized particles were preincubated overnight with IgG (0.1 mg IgG/mL at 4°C) to saturate nonspecific binding sites. Bio-Gel is frequently used as packing matrix in perfusion systems, while monosized plastic particles have not been used before for this purpose.

The German astronomer Johannes Kepler deduced in 1611 that the relative volume of spherical particles packed in the tightest hexagonal pattern is $\pi/\sqrt{18}$ or approximately 74% of the total space occupied. The monosized particles will sediment and pack in the densest hexagonal packing pattern with some disorder at the edge against the chamber wall. The cells will pack in between the approximately 8–10 times larger particles in the pyramid-shaped spaces formed between neighboring (four) particles (Figure 3).

In experiments using the prolactin-secreting GH₄C₁ cells, the number of cells per column was $50\text{--}80 \times 10^6$. The total perfusion chamber volume was 0.5–0.7 cm³, and the total cell volume, assuming an average cell diameter of 15 μm , was then about 10% of the chamber volume.

Measurements of PRL

The concentration of PRL in the column eluate was measured by radioimmunoassay (4,13). All samples from one perfusion experiment were included in the same assay series. Since the sample volume was only 100 μL , a single measurement of each sample was performed. The intra-assay CV was less than 6% ($n = 30$), and the inter-assay CV was 10%–11% ($n = 30$) (13). The limit of detection was 0.3 ng/mL, corresponding to a secretion rate of 0.45 ng/min.

RESULTS

Flow Characteristics of the Perfusion System

To study the flow characteristics of a square pulse perfused through the perfusion chamber using the two different matrix materials (monosized particles or Bio-Gel P6-DG particles), we compared the perfusion profiles of a low molecular weight substance, ¹²⁵I (125 g/mol) and a peptide of fairly high molecular weight, ¹²⁵I-labeled prolactin (22 000 g/mol). The step-like stimulation pulse lasted 30 fractions (3.0 mL) or 120 s at a flow rate of 1.5 mL/min. The chamber volume was 0.5 cm³ for both matrix materials, and no cells were included.

Figure 4A presents the perfusion profiles of ¹²⁵I and ¹²⁵I-prolactin for both monosized particles and Bio-Gel P6-DG particles. Each of the four profiles is based on a series of 10 perfusions, and the data are given as mean values $\pm$ SEM. The arrow indicates the real time when the valve looping the test solution (¹²⁵I or ¹²⁵I-prolactin) was switched to perfuse in the direction of the chamber. The delay before the radioactive marker appeared in the eluate is mainly a reflection of the dead space in the system (afferent tubing, perfusion chamber and efferent tubing).

Using Bio-Gel as cell-supporting matrix, the perfusion curves of ¹²⁵I-prolactin and ¹²⁵I were clearly different. Detection of ¹²⁵I in the eluate (>5% of the infusion concentration) was delayed by 3 fractions (12 s) compared to detection of the larger ¹²⁵I-prolactin. However, the perfusion curves merged after about 10 fractions, and the eluate concentration of both substances reached 95% of the infusion concentration after approximately 15 fractions (1 min) or 1.5 mL perfusate. A similar time shift was observed at the termination of the perfusion pulse. Compared with the larger (175 times) ¹²⁵I-prolactin, the initial decline of the eluate concentration of ¹²⁵I was delayed by 3 fractions.

In contrast to the results obtained with Bio-Gel as cell-supporting matrix, the ¹²⁵I and ¹²⁵I-prolactin perfusion profiles were identical using the monosized particle column, indicating that the flow of various compounds through the system is independent of molecular size. The eluate concentration of ¹²⁵I-prolactin and ¹²⁵I started to rise at the same fraction (fraction 1) and reached 95% of the infusion concentrations in fraction 6 (after 24 s). The two falling curves at the termination of the perfusion pulse were also identical.

The perfusion profile obtained with the monosized particle matrix is thus a much better approximation to the perfused square pulse than the profiles obtained with the Bio-Gel matrix. The perfusion profile is also independent of the molecular weight of the perfused substances, which is an essential feature when comparing the secretion kinetics of substances differing in molecular size.

Figure 3. Monosized particles and stacked cells. Scanning electron micrograph (EM) of hexagonally packed monosized particles with cells in the interparticle spaces.

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Figure 4B presents a simplified perfusion profile showing the main flow characteristics of the monosized particle matrix. The rising and falling slopes of the profiles are identical, consisting of an initial phase with a constant rate of rise or decay (Figure 4B, a and b) and a later phase with a gradually declining rate of concentration change (Figure 4B, c and d). The areas between the indicated square pulse (square box) and the inclining and declining phases of the perfusion profiles are caused by blunting of the initially sharp front and tail of the step-like perfusion pulse. This is a result of mixing and dilution caused by inevitable differences in flow velocity in central and peripheral layers in tubes with laminar flow, in addition to turbulence and non-specific binding in the perfusion system. Turbulence is likely to occur in areas with steep changes in flow velocity due to the altered cross-sectional area of the system. To reduce this effect, the inlet and outlet zones of the chamber are cone shaped, making the velocity changes more gradual.

In experiments using GH₄C₁ cells, the initial cell loss from the packed perfusion chamber was 0.5%–2% of the total cell number during the first 10 fractions. Cells were not detected in lat-

er eluate fractions. The total cell death (monitored with trypan blue) was 20%–30% after a 2-h perfusion. Approximately 67% of the dead cells were destroyed during packing.

Kinetics of Secretagogue-Induced PRL Secretion from GH₄C₁ Cells

To demonstrate the potentials of the perfusion system, we present examples of secretion data from GH₄C₁ cells (these studies have previously been published in more detail elsewhere). The profiles of PRL secretion induced by TRH and vasoactive intestinal peptide (VIP) are compared in Figure 5. The column matrix used was the monosized particles. The square boxes show the period of stimulation, compensated for the delay due to dead space. TRH (panel A) induced exocytosis of PRL within the first fraction or 4 s at the employed flow rate of 1.5 mL/min. The secretion profile has an initial 8–13 fraction phase (30–50 s) with an increase of 2–5 times in prolactin secretion relative to the basal release rate, followed by a prolonged second phase with a near doubled rate of PRL secretion. The instant first-phase release corresponds to a rapid IP₃-induced release of intracellular Ca²⁺ (5).

During the prolonged second phase secretion, the cells display an increased frequency of Ca²⁺-dependent action potentials that elevates the cytosolic Ca²⁺ concentration and induces exocytosis of PRL. This TRH-induced second-phase release is mimicked by the tumor-promoting phorbol ester, TPA (23). The peptide bombesin induces a PRL-secretion profile indistinguishable from that obtained with TRH (5). The peptides VIP and PHI (peptide with N-terminal histidine and C-terminal isoleucine), on the other hand, induce a monophasic release pattern with a delay of about 60 s (panel B). This release is associated with elevation of intracellular cAMP concentration (3).

DISCUSSION

The time resolution of a perfusion system is proportional to the fractional volume divided by the flow rate. The minimal size required of the fractional volume depends on the concentration of the studied product necessary for detection by the assay used. The concentration of secreted product depends on the volume of cells or vesicles in the perfusion chamber and on the amount of product secreted from each cell or

Figure 4. Perfusion system flow characteristics. Arrows indicate real time of stimulation. The chamber was perfused with step-like pulses (square box) of the test solutions. The distance between the arrow and the start of the ascending slope represents dead space volume between perfusion media and fraction collector. (A) ¹²⁵I (circles) and ¹²⁵I-prolactin (PRL) (squares) perfusion profiles for Bio-Gel P6-DG (BG) matrix (open symbols) and monosized particles (MS) (Dynospheres® EXP-SS-78.3) (filled symbols). Data are given as mean values ± SEM (*n* = 10). (B) Simplified perfusion profile for the monosized particle matrix; a and b, phases with constant rate of rise and decline of eluate concentration; c and d, phases with gradually decaying rate of eluate concentration change. The areas between the indicated step-like test pulse (square box) and the inclining and declining profile segments are a result of mixing, dilution and nonspecific binding in the system.

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vesicle after stimulation. In a perfusion chamber, higher cell or vesicle volume will give a higher flow resistance and increase the danger of cell or cell-supporting matrix collapse at high flow rates. Also, the time resolution depends on the kinetics of solution exchange within the perfusion chamber.

The time resolution of a given system can be optimized by (i) increasing the sensitivity of the assay used to measure the secreted product; (ii) reducing the volume of each fraction; (iii) increasing the flow rate of the perfusion system; (iv) increasing the total cell volume and the amount of secretion product; (v) increasing the ratio between cell volume/dead space and (vi) optimizing the flow kinetics in the perfusion chamber. Several perfusion systems with high time resolution have previously been described.

Subsecond Time Resolution

Pearce and co-workers (24) have described a superfusion system for study-

ing the release of preloaded $^{22}\text{Na}^+$ and $^{45}\text{Ca}^{2+}$ from purified bovine rod outer segment disks. About 200 μg disk membrane vesicles, preloaded with $^{22}\text{Na}^+$ or $^{45}\text{Ca}^{2+}$, were retained in a fiberglass filter sandwiched between a pair of nitrocellulose filters in the 6-mm diameter superfusion chamber with a dead volume of approximately 20 μL . The cell protein content was only about 2% of that contained in our system. With a flow rate of 0.5 mL/min, the calculated exchange within the superfusion compartment was over 90% complete within 480 ms. Release of cations in response to exposure to cyclic nucleotides was measured by collection of the superfusate at 25-ms intervals.

Turner et al. (31) have described a superfusion system with cells or synaptosomes entrapped in fiberglass filters. The chamber volume was less than 0.02 cm^3 and the volume of cell material was around 0.01 cm^3 . The flow rate was 1.5 mL/min. Prelabeling of the secretory product with $[^3\text{H}]$ made it possible to accept smaller fraction volumes

compared with our system, giving a time resolution of 50–60 ms.

Using isolated nerve terminals (synaptosomes) immobilized in Sephadex® gel inside a disk-shaped perfusion chamber, Verhage et al. (33) achieved a time resolution of 600 ms. The relatively large cross-sectional area of the disk-shaped chamber secured a sufficiently low flow resistance to allow the required flow rate with an acceptable pressure gradient. The maximum flow rate was 10 mL/min. The cell material was thus confined to a thin layer, as opposed to the columnar packing in our system. The perfusion chamber volume was approximately 0.04 cm^3 , which is 5.6% of the average perfusion chamber volume of our system. Their method is therefore limited to small volumes of cell material.

Subminute Time Resolution

Stojilkovic et al. (27) studied luteinizing hormone (LH) secretion from rat anterior pituitary cells. The cells

Figure 5. TRH- and VIP-induced prolactin secretion profiles. (A) Prolactin (PRL) secretion from approximately 60 million perfused GH_4C_1 cells in response to a 120-s stimulation with 100 nM TRH (square box). (B) Comparable secretion profile in response to 100 nM VIP. Samples were collected every 4 s. Arrows indicate real time of stimulation. Flow rate, 1.5 mL/min, chamber volume, 0.7 cm^3 .

were incubated with preswollen Cytodex®-1 beads and loaded into a 0.5-mL perfusion chamber. The number of cells per chamber was $15\text{--}20 \times 10^6$, and fractions were collected every 5 s. The perfusion profile of [^{125}I]-gonadotropin-releasing hormone (GnRH) showed an exponential rise with a half time of 45 s. The maximum value of 100% of the original [^{125}I]-GnRH concentration was reached after 2.4 min and was followed by an exponential fall during the wash-out period. Flow rate and fraction volume were not reported in this article. However, in other publications from this group (15,17,34), flow rates and fraction intervals of 0.17–0.6 mL/min and 15 s to 1 min were used.

Drejer et al. (10) studied the release of preloaded [^3H]-GABA from a monolayer of mouse cerebral cortex interneurons in a 30-mm dish. The dead volume of the system was 200 μL , the flow rate was 2 mL/min and fractions were collected every 30 s. A complete change of extracellular medium was obtained within 5–10 s. By using an open perfusion system, potentially damaging pressure gradients were avoided. However, the volume of monolayered cells in a 30-mm diameter dish is only about 1% of the cell volume used in our perfusion system.

Dionne et al. (9) studied insulin secretion from isolated rat pancreatic islets. The chamber volume was 40 μL , the flow rate was 0.95 mL/min and the fraction interval was 18 s. The total islet volume was less than 1% of the average cell volume used in our experiments with GH_4C_1 cells.

Yoshimura and Nezu (36) studied amylase release from isolated rat parotid cells by using a column perfusion system with a Bio-Gel P-2 matrix. The perfusion chamber consisted of a 1-mL pipet, the flow rate was 1 mL/min and fractions were collected every 30 s.

Yao et al. (35) have developed a perfusion system for isolated rat islets. Approximately 100 islets of similar size were hand picked under the microscope and placed in the perfusion chamber consisting of a 1-mL plastic syringe with two layers of 10- μm nylon mesh net at the bottom. The perfusion rate was 0.36 mL/min, and the fraction interval was 30 s.

Brand et al. (6) have described a perfusion system for studying cellular me-

tabolism by continuous monitoring of the perfusate pH. They used HeLa cells attached to nonporous glass beads with 170–210 μm diameter ($7\text{--}11 \times 10^4$ HeLa cells per chamber). The authors argued that (6) “nonporous, hollow glass beads were chosen for cellular attachment rather than more conventional porous support matrices, such as Sephadex (Amersham Pharmacia Biotech), Bio-Gel (Bio-Rad Laboratories) and Cytodex (Amersham Pharmacia Biotech) because solid glass beads reduce dead volume within the cell chamber and eliminate molecular separation by gel sieving.” This is in accordance with the data we obtained when comparing Bio-Gel and solid, monosized particle matrices in our own system.

Our perfusion system offers several advantages compared to the static incubation of endocrine cells in studies of the control of hormone secretion. The similarity to the *in vivo* situation regarding continuous removal of the secretory products precludes product accumulation and possible feedback effects on the hormone-secreting cells. The continuous perfusion also makes it possible to study the effects of pulsatile stimulation comparable to the *in vivo* situation (11). Compared with static incubation systems, perfusion furthermore allows a time-course analysis of the cellular responses with improved time resolution.

Stachura (26) emphasized two important advantages of perfusion systems. First, the response to a given agent can be fingerprinted to show the latency of the initial and the maximal effects, the persistence of the effect after removal of the stimulus and also possible post-stimulatory phenomena. Second, a minimal response will not be lost within the bulk of the ongoing basal release, and oscillating responses will not be concealed. The high time resolution provided by our system also makes it possible to compare the time-course of hormone secretion with the time-course of activation of alternative transmembrane signaling systems.

The major improvement of our perfusion system compared with previous versions is the use of solid monosized particles as the cell-supporting matrix. The solid, nonporous nature of the particles makes the effective extracellular

distribution volume within the perfusion chamber equal for all compounds, irrespective of their molecular size. This means that the secretagogue and the secretory product, which often differ considerably in molecular size, are transported in the same direction at the same flow speed. Fast secretory responses to step-like secretagogue pulses will consequently not be blurred because the initial release products from cells at different levels in the perfusion chamber will reach the outlet at the same time. Because the perfusion profiles are identical for compounds of different molecular size, it is also possible to compare the secretion kinetics of two or more products simultaneously released in the same cell column. The eluate fractions may then be divided for two or more separate assays. Alternatively, the released products may be radiolabeled with tracers exhibiting either beta or gamma radiation and counted independently.

A wide size range of the particles in the chamber matrix will inevitably cause the smaller particles to pack between the larger ones, which leads to uneven and obstructed flow. However, the size variation of the monosized particles is only about 5%, which gives a nearly homogeneous flow through the perfusion chamber and a minimum flow resistance. It is therefore possible to obtain a high flow rate with an acceptable pressure gradient in the perfusion chamber, thus avoiding mechanical destruction of the cells. Increasing the flow rate will improve the time resolution, but it will also increase the pressure gradient in the perfusion chamber. Increasing the number of cells in the chamber to increase the hormone concentration in the eluate also leads to increased flow resistance and an elevated pressure gradient in the chamber.

The monosized particles do not readily deform when exposed to pressure stress. This is advantageous because changes in shape would reduce the volume between the particles and damage the packed cells. A change in particle shape would also increase the flow resistance when the flow rate is increased. The use of a less stable matrix material like Bio-Gel makes it more likely that the pressure gradient will deform the particles and induce higher flow resistance in the column. The

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increased pressure gradient, which is necessary to obtain constant flow rate, will further degrade the matrix, and this vicious circle will continue until the column is clogged.

Comparing our perfusion system with those previously described (provided the time resolution is in the same range), a major advantage of our system is the potential of using a much larger number of cells or vesicles for each experiment. This is advantageous in studies of secretion with a high time resolution in cells or vesicles with low basal secretion rates or when using assays with limited sensitivity.

In our studies of PRL secretion from GH₄C₁ cells, we were unable to obtain a time resolution below 4 s. However, it would be desirable to improve the time resolution of the system further for more detailed study of the secretion kinetics. A cell column with approximately 50 million GH₄C₁ cells and a

monosized particle matrix resisted a perfusion flow rate of 6 mL/min without collapsing or losing cells. Having a cell line with a higher product secretion rate or a more sensitive product assay, the perfusion system could provide a 1-s time resolution using a flow rate of 6 mL/min and a fraction collection volume of 50 µL.

In conclusion, we have developed a perfusion system optimized for high flow rate and high time resolution. The core of the system is a matrix of solid, monosized particles that support the cells and is able to withstand the necessary pressure gradient without deformation. The perfusion profile of a substance is independent of its molecular size. Using the high time resolution of our perfusion system, we have been able to fingerprint different patterns of prolactin secretion from GH₄C₁ cells, depending on the secretagogue used. The differences in the secretion profiles

for TRH and VIP are probably related to the kinetics of the different transmembrane and intracellular mechanisms involved (3,5).

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