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Video Article

Mechanisms Underlying Gut Hormone Secretion Using the Isolated Perfused Rat Small Intestine

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Abstract

The gut is the largest endocrine organ of the body, producing more than 15 different peptide hormones that regulate appetite and food intake, digestion, nutrient absorption and distribution, and post-prandial glucose excursions. Understanding the molecular mechanisms that regulate gut hormone secretion is fundamental for understanding and translating gut hormone physiology. Traditionally, the mechanisms underlying gut hormone secretion are either studied in vivo (in experimental animals or humans) or using gut hormone-secreting primary mucosal cell cultures or cell lines. Here, we introduce an isolated perfused rat small intestine as an alternative method for studying gut hormone secretion. The virtues of this model are that it relies on the intact gut, meaning that it recapitulates most of the physiologically important parameters responsible for the secretion in in vivo studies, including mucosal polarization, paracrine relationships and routes of perfusion/stimulus exposure. In addition, and unlike in vivo studies, the isolated perfused rat small intestine allows for almost complete experimental control and direct assessment of secretion. In contrast to in vitro studies, it is possible to study both the magnitude and the dynamics of secretion and to address important questions, such as what stimuli cause secretion of different gut hormones, from which side of the gut (luminal or vascular) is secretion stimulated, and to analyze in detail molecular sensors underlying the secretory response. In addition, the preparation is a powerful model for the study of intestinal absorption and details regarding the dynamics of intestinal absorption including the responsible transporters.

Video Link

The video component of this article can be found at https://www.jove.com/video/58533/

Introduction

The gut is the largest endocrine organ of the body, producing more than 15 different peptide hormones that regulate nutrient absorption and nutrient disposition, intestinal growth and modulate appetite[1]. Gut hormones are, therefore, involved in many fundamental physiological processes, and understanding these patterns of secretion and the molecular details that control secretion of the respective hormones is thus important for our basic physiological understanding and for addressing translational aspects of gut hormone actions; but how can one study the molecular sensing mechanisms underlying gut hormone secretion? In general, hormone secretion can be studied in intact organisms (humans or experimental animals), from isolated gut preparations or from gut hormone-secreting primary cell cultures or immortalized cell cultures[2,3,4,5,6]. Our preferred model is the isolated perfused rat small intestine, which is a physiologically relevant model that allows the secretion of gut hormones to be studied in detail with optimal time resolution (secretion rates can be determined with any time base down to the second), and the results are likely transferrable to an in vivo situation[7]. Here, we provide a detailed protocol on how to perform this procedure, but first we will discuss other methods for studying gut hormone secretion, including the benefits and limitations of these models compared to the isolated perfused rat small intestine.

If one wishes to establish whether a specific compound regulates the secretion of certain gut hormones, studies in humans are the ultimate goal. Thus, if a compound shows great effects on the secretion of one or several gut hormones in rodents (in vivo or perfusions) or from hormone secreting cells (cell lines or primary cells), this effect is only relevant to medicine and human physiology if it can be confirmed in humans. However, there are clear limits for the type of studies that can be performed in humans, and in vivo studies on experimental animals are, therefore, often the second-best option for such studies. Mice and rats are the most frequently used experimental animals presumably due to their convenient size, low cost and the option to genetically alter the genes suspected of being involved in the specific study questions (e.g., knock out of a certain transporter or receptor). In general, in vivo models benefit from being physiologically intact, but also have several limitations. Most importantly, the small size of rodents, particularly mice, is a limiting factor, as most assays for gut hormone quantification require at least 20 µL of plasma (and often much more), meaning that at least 100 µL of blood has to be withdrawn to make a duplicate quantification. Therefore, it is only possible to obtain very few samples corresponding to baseline samples and one or two post-stimulation samples (the total blood volume in a mouse of 20 g is ~1.4 mL). Consequently, potential secretory responses (e.g., rapidly or late-occurring responses) may therefore be missed.

In the perfusion model, this issue is overcome, as large sample volumes are obtained (flow rate: 7.5 mL/min) and the collection intervals can be adjusted as needed to ensure that the rapid and short-lasting responses are not missed (we collect samples every min)[7]. Another issue with in vivo studies in rodents is that most gut hormones are even more rapidly eliminated or metabolized than in humans[8,9,10], which may complicate the subsequent biochemical analysis. For instance, we showed that GLP-1 is metabolized in mice at an even faster rate than in humans (where $T_{1/2}$ is 1-2 min[11]) and, more importantly, that the cleavage of GLP-1 in mice involves, in addition to N-terminal cleavage by dipeptidyl-peptidase-4 (DPP4) (which is the major GLP-1 degrading enzyme in humans), further cleavage by the enzyme neutral endopeptidase 24.11[12]. Consequently, current commercial assays for the quantification of GLP-1, which are either based on the intact isoform of GLP-1 (7-36amide) or the DPP-4 cleaved isoform (9-39amide), vastly underestimate GLP-1 secretion in mice and result in misleading results[12]. In the isolated perfused rat small intestine, most of the metabolism of secreted hormones is eliminated or markedly reduced, since plasma-mediated degradation is avoided, and liver/kidney/lung extraction/degradation is prevented (because perfusate is collected as it leaves the gut).

Of course, important insight can be generated by the use of genetically modified animals, e.g., sodium-glucose transporter-1 knockout mice[13], but a detailed assessment of the molecular sensors involved in secretion often requires consideration of multiple molecular sites, ranging from molecular transporters to ion channels and from different G-protein-coupled receptors to intracellular proteins. For instance, we targeted the activity of nine different molecular sites when unraveling the molecular sensors responsible for glucose-stimulated GLP-1 secretion[7]. A similar investigation would not be possible in vivo as some of the compounds used have unspecific or harmful/lethal effects. For instance, when using the perfused gut, it was possible to assess the role of intra-cellular glucose metabolism for the secretion of GLP-1 and neurotensin by blocking ATP formation with 2-4-dinitrophenol[7,14] as well as the role of voltage-gated calcium channels for bile acid stimulated GLP-1, NT and PYY secretion[3]. Indeed, the highly toxic sodium channel blocker tetrodotoxin can be successfully applied in the perfusion studies. Finally, in the perfusion model it can be directly assessed where in the gut a certain compound stimulates secretion of a certain hormone, as the investigator can simply choose and prepare the desired region to perfuse, and at the same time it can be investigated whether a stimulus causes secretion by activation of molecular sensors from the luminal or vascular side of the intestine[3,15,16].

The secretory mechanisms underlying gut hormone secretion may also be studied by use of gut tissue pieces (including human tissue), primary intestinal cultures (usually from mice), immortalized hormone secreting cell lines (of mouse or human origin), by gut tissue mounted in Ussing chambers or by organoids (both most often from mice)[2,3,4,5,6,17,18]. Compared to intestinal perfusions, studies on human gut pieces, primary cell cultures and cell lines are technically easier to perform and are a faster and cheaper way of generating data, but of course the study of gut pieces requires access to fresh human gut specimens. However, in these models the normal cell polarization of the gut is inherently lost, meaning that these models cannot be used to assess normal activation of molecular sensors, and absorption processes also cannot be studied. Moreover, such studies usually employ static incubations (for up to several h) which is highly non-physiological and has nothing to do with the cells' normal secretory dynamics, because the secreted product is not removed and thus may feedback influence the secretion of hormones. In contrast, in the perfused intestine, secreted and absorbed molecules are efficiently removed by the mucosal microcirculation as they are in vivo, ensuring that the transmucosal gradients are maintained so absorption and secretion can occur at a normal rate. Furthermore, cell cultures may have dedifferentiated from their native enteroendocrine cell origin, meaning that they are no longer representative of the native cells in terms of peptide content and expression of molecular sensors, although they may still secrete the hormone in question. This is, for instance, the case for GLP-1 secreting cell lines[19].

It is, therefore, our opinion that primary cell cultures or cell line studies are most suited for screening purposes and for performing types experiments that cannot be performed in vivo or in the isolated perfused gut. For instance, a true strength of the primary cell cultures and cell line cultures is that intra-cellular secondary messengers (e.g. Ca^{2+}, cAMP, NAD(P)H) can be monitored in real time, and electrical signaling of the hormone secreting cells can be investigated[20,21,22]. In addition, siRNA knockdown can be done, which is particularly useful if specific inhibitors are not available[20,21,22,23,24]. Gut tissue from mice mounted in Ussing chambers has recently been used for studying the molecular mechanisms underlying bile-acid stimulated GLP-1 secretion, while intestinal organoids (from mice) and human gut pieces have also been used for studying the molecular details of gut hormone secretion[17,25]. Whereas the former benefits from being polarized[2] all of these models involve static incubations. Studies on human gut pieces, however, benefit from using human, rather than rodent, tissue which is important since species difference in tissue expression of 7TM receptors and molecular transporters may result in different molecular sensing pathways between species. In fact, most data in this field has been generated by studies on either pigs, mice or rats, and it remains elusive whether these findings can be transferred to humans. It is, however, reassuring that the molecular sensing mechanisms that underlie glucose-stimulated GLP-1 secretion appear to be similar between mouse, rat, man, and transcriptomic and peptidomic profiling of mouse and human L-cells revealed strong global similarities between the two species[7,18,26,27].

The isolated perfused rat small intestine, however, also has some limitations that should be considered. Most importantly, it is impossible to determine whether a given secretory response results from direct activation by the test substance of the targeted hormone-producing cells or rather is caused by an indirect mechanism. For instance, KCl instantly increases GLP-1 secretion from the perfused intestine[7], but it remains unknown whether this is a consequence of direct depolarization of the L-cell or results from depolarization of neurons close to the L-cells or effects of simultaneously released paracrine stimulators/inhibitors. Data arising from studies using the perfused intestine which aim to elucidate the molecular mechanisms underlying secretion should, therefore, always be put into context with data obtained from other more specific models to increase the ability to establish causality. For instance, glucose-stimulated GLP-1 secretion from the GLP-1 secreting cell line GLUTag[28,29] and from primary mouse L-cells depend on the activity of the glucose transporters (SGLT1 and GLUT2). Blocking these transporters in the perfused rat small intestine also attenuates secretion[20], meaning that it is likely that glucose-stimulated GLP-1 secretion is largely mediated by direct actions of glucose on the L-cell. Another important limitation of the isolated perfused intestine is that some of the lipids are difficult to study due to their hydrophobicity. Although it is possible to investigate the final products of lipid digestion (fatty acids, diacyl glycerols, lysophosphatidylglycerols, etc.) and although the preparation may be able to re-esterify the lipids intra-cellularly and perhaps pack them into chylomicrons, the transport of the chylomicrons out of the cells and their subsequent uptake by the lacteals of the villi is disrupted, since the lymph flow in the isolated gut cannot be secured. Most likely, therefore, lipid absorption is halted once the absorbed products start to accumulate in the cells. The in vitro cell systems are even less suitable for lipid studies because of their lack of polarization. Obviously, this limitation is only relevant for lipids that are absorbed and transported via the lacteals, whereas those absorbed via the intestinal blood vessels are likely to be handled normally.

Protocol

All studies were conducted with permission from the Danish Animal Experiments Inspectorate (2013-15-2934-00833) and the local ethical committee, in accordance with the guidelines of Danish legislation governing animal experimentation (1987) and the National Institutes of Health (publication number 85-23).

1. Experimental Animals

1. Obtain male Wistar rats (250 g) and house 2 per cage, with ad libitum access to standard chow and water, and maintain in a 12:12 h light-dark cycle. Our animals facilities uses non-treated water and Alrtromin rodent chow (1319 FORTI, Brogaarden, Lynge, Denmark).
   Note: Allow animals at least one week of acclimatization.

2. Preoperative Preparations

1. Make perfusion buffer: Krebs-Ringer bicarbonate buffer supplemented with 0.1% BSA (fraction V), 5% dextran T-70, 3.5 mmol/L glucose, and 5 mmol/L pyruvate, fumarate, and glutamate (if needed); pH 7.4.
2. Prepare an adequate amount of perfusion buffer by filtering it through an appropriate size of filter and adjust pH to ~7.5 by dropwise addition of 5 M HCl.
3. Add 3-isobutyl-1-methylxanthine (IBMX) (if needed) directly to the buffer on the day of the study to a final concentration of 10 µM.
   NOTE: IBMX is a phosphodiesterase inhibitor that increases $[cAMP]_i$ and thereby restores the sensitivity and responsiveness of the gut in terms of secreting hormones to a secretory stimulus.
4. Prepare test solution(s). Prepare vascular stimuli at a 20x higher concentration than the desired final concentration in filtered and pH-adjusted perfusion buffer. Prepare luminal test stimuli in isotonic saline in the final concentration.
5. Dissolve not readily soluble compounds in 100% dimethyl sulfoxide (DMSO) and dilute further in perfusion buffer or isotonic saline. For vascular stimuli, keep the final DMSO concentration at 1% or below, as concentrations above this damage the intestine and may lead to unspecific gut hormone secretion. Measure pH and adjust to ~7.5 if needed.

3. Operation and Perfusion

NOTE: An illustration of a perfusion setup is provided in **Figure 1**.

1. Anesthetize the rats by use of an anesthetic regimen that can sustain surgical anesthesia and analgesia for 30-40 min by Hypnorm/Midazolam (0.3 ml/100 g body weight, per ml: Hypnorm: 0.08 mg fentanyl, 2.5 mg fluanisone, 0.45 mg Methyl Parahydroxybenzoate, 0.05 mg Propyl Parahydroxybenzoate, Midazolam: 1.25 mg, Matrix Pharmaceuticals, Hellerup, Denmark)
2. Check for lack of reflexes (toe pinch), place the rat on the heated operating table and perform an incision of the skin to expose the intestine.
3. **Expose the terminal part of the colon by moving the small intestine aside as much as possible. Tie of supplying vasculature to the colon and excise it gradually starting from the terminal part of the colon, moving towards the small intestine.**
   1. If only a certain part of the small intestine is required for perfusion (the upper half), excise non-required segments after tying off supplying vasculature. To minimize tissue damage, moisturize the intestine with isotonic saline and use swabs to remove connective tissue.
4. Insert a plastic tube (length: ~15 cm, outer diameter: ~0.4 cm) into the intestinal lumen (in the proximal part) and tie it properly using sutures. Flush carefully with isotonic saline (room temperature) to empty the lumen of chyme.
5. Perfuse the lumen with the isotonic saline at a flow of 0.25 mL/min, using a 10 mL syringe connected to a syringe pump.
6. Move the intestine aside so the upper mesenteric artery is accessible and remove connective and fat tissue to expose the artery.
7. Place two sutures under the mesenteric artery by using fine point forceps; one of the sutures is for lifting the artery to control bleeding once the artery has been cut and the other is for securing the catheter that is to be placed in the artery. Take caution not to perforate the artery.
8. Place two sutures under the portal vein for securing the metal catheter that is going to be placed in the vein for collection of perfusion effluent.
9. Before cutting the hole in the mesenteric artery, ensure that the catheter that is going to inserted into the artery is filled with perfusion buffer to prevent air embolus formation.
10. Cut a small hole in the mesenteric artery using a pair of surgical scissors and insert the plastic catheter immediately after.
11. Immediately after the catheter has been secured, initiate perfusion of the gut by starting the roller pump (flow rate = 7.5 mL/min).
12. Confirm that the blood vessels in the gut turn pale within few seconds, and the portal vein turns pale.
13. Immediately after proper perfusion has been established, cut a hole in the portal vein, insert the metal catheter and secure it with the suture.
    NOTE: The catheter can be difficult to place but lifting the vein up by cautiously pulling the most proximal suture helps.
14. Once the catheters are in place and perfusion output is satisfactory, kill the rat by diaphragm perforation, being careful not to rip out the catheters.
15. Collect perfusion output for 1 min and measure the volume. Start the perfusion pressure acquisition/recordings by click **Execute Experiment** in the pressure recording program.
16. Cover the gut with moistened tissue to prevent it from drying out during the experiment.
17. Ensure that the distal end of the intestine is not blocked so that the luminal effluent can exit, otherwise the intestine will swell, edema will develop, perfusion pressure will increase, and perfusion output will drop.
18. Leave the preparation for approximately 30 min before initiating the experiment.
    NOTE: The secretory outputs of gut hormones are very unsteady for the first 15 min of perfusion, so this equilibration step is needed to get a steady baseline.

4. Experiment

1. After approximately 30 min of perfusion, start the experiment by collecting the first baseline sample using a fraction collector. Collect samples at the desired time interval (e.g., every min, 6.5-7.5 mL is usually collected) and place them on ice within few min.
2. **Inspect the bubble trap regularly and, if emptied, refill it with perfusion buffer.**
    1. Collect the buffer through the three-way cock-valve immediately before it enters the organ and from the catheter inserted in the portal vein (after it has been perfused through the organ) to confirm that the organ is metabolically active. Collect samples at the start and end of experiment to assess viability throughout the experiment.
    2. Measure samples quickly, with an automated blood-gas analyzer, as most plastic contains/syringes are not completely airtight, giving rise to exchange with the atmospheric air.
3. After 10-15 min of baseline collection, stimulate with the first test substance. Administer intra-arterial stimulations with a syringe pump through a three-way stopcock (flow = 0.350 mL/min).
4. Perform luminal stimulations by an initial bolus injection (2.5 mL/min over the first 5 min), to replace the isotonic saline that is already in the lumen, followed by administration of the test solution a lower flow rate (0.5 mL/min).
5. To ensure that the lumen is quickly emptied of test substance once the luminal stimulation period is over, flush the intestinal lumen with isotonic saline by applying same flow rates as above.
6. Collect baseline samples for 15-30 min before the next test substance is administered. Depending on the protocol, 2-3 test stimuli can usually be included per experiment. If the experimental protocol includes activation/inhibition of a given molecular site simultaneously with administration of a given secretagogue, always pre-stimulate with the activator/inhibitor 10-15 min before the administration of the secretagogue to ensure that the molecular site is inhibited at the very beginning of secretagogue infusion.
7. At the end of the protocol, administer (5-10 min) a suitable positive control to test for responsiveness of the preparation and collect 10-15 min baseline samples after the stimulation period.
   NOTE: Several test substances can be used as positive control, e.g., stimuli for increasing $[cAMP]_i$ (e.g., foskolin or IBMX), $[Ca^{2+}]_i$ (e.g., bombesin or neuromedin C), depolarizing agents (e.g., 30-50 mM KCl) or more physiologically relevant stimuli such as macronutrients: glucose, peptones, amino acids, etc. However, the choice of positive control depends on the experimental outcome. Glucose is for instance a poor cholecystokinin (CCK) secretagogue whereas bombesin is not a robust secretagogue for glucose-dependent insulinotropic peptide (GIP) secretion[30].
8. After the end of experiment, excise the perfused gut, weigh it and measure its length. Put it in paraformaldehyde and store it (4 °C) for potential later histochemical analysis of tissue integrity/damage, for instance by H&E staining.

5. Biochemical Measurements

1. Quantify concentrations of secreted peptides in the venous effluent by use of an in-house or commercially available radioimmunoassays (RIA) or ELISA-assays. For studies that investigate intestinal absorption, quantify the molecule of interest, e.g., glucose or amino acids, in the venous effluent.
   NOTE: The perfusate is usually a good basal buffer for most analyses, including assays that relies on enzymatic reactions (such as quantification of glucose by the glucoseoxidase method).

6. Data Analysis

1. Present data in different ways, for instance as a time-concentration graph showing the actual secretory output (flow x concentration) and as column plot depicting the total secretory output during baseline and response periods (usually 10-15 time points).
2. Plot the actual measured concentration of the respective hormones as concentration units. (pmol/L),
   NOTE: It is preferable to instead express data as output (fmol/min) because with this model, in contrast to in vivo studies, the obtained values are the actual secretory output (because of the constant collection of perfusion effluent).
3. Depending on the number of groups to be analyzed statistically, use two-tailed paired t-test (two groups) or one-way ANOVA for repeated measurements (more than two groups) followed by an appropriate post-hoc test for multiple comparisons.

Representative Results

The ability to determine whether a given stimulus causes secretion of the gut hormone of interest relies on a steady baseline secretion. Furthermore, if no response to the stimulus is observed, a robust secretory response to the positive control must be evident to exclude that the lack of response to the test stimulus does not reflect a general lack of responsiveness. **Figure 2A** and **2B** shows an example of good quality data; GLP-1 secretion from the isolated perfused rat small intestine are shown at 1-min intervals (means ± SEM). At basal conditions secretion is steady; both before and after administration of the test stimulus (insulin), and a robust secretory response to the positive control (bombesin (BBS)) is evident. In addition, averaged GLP-1 secretion at basal conditions (during the respective baseline periods preceding insulin or BBS administration) as well as averaged secretion during insulin and BBS stimulation are shown as bar graphs (means ± SEM). Secretion was tested for statistical significance by one-way ANOVA for repeated measurements. Based on these combined data, it can be concluded that intra-arterial insulin does not stimulate GLP-1 secretion from the isolated perfused rat small intestine.

Figure 2C and **2D** shows an example of GLP-1 secretion from the isolated perfused rat small intestine. Unlike **Figure 2A** and **2B**, these data are of poor-quality; the secretion is unsteady at basal conditions and drifts upwards throughout in a manner that appears to be unrelated to test substance administrations (intra-luminal and intra-arterial fructose, respectively) and the positive control, BBS, does not result in statistical significant increased GLP-1 secretion. It is, therefore, impossible to conclude whether the fructose stimulations give rise to GLP-1 secretion, e.g., is the increased GLP-1 secretion at the end of the vascular fructose stimulation a fructose-mediated response or not.
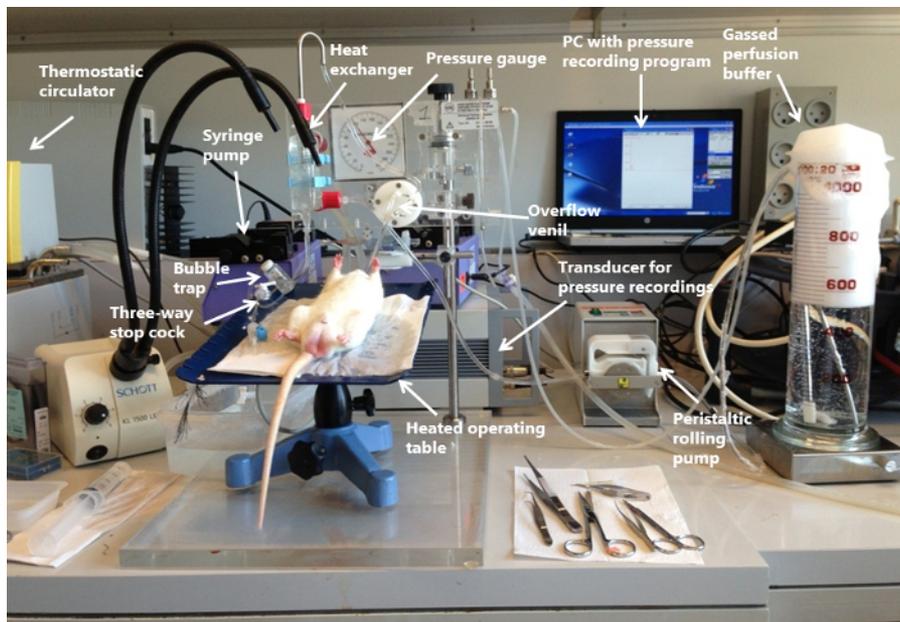
Figure 1: An example of a perfusion setup. The system consists of a plexiglass stand, a heated operating table, a heat exchanger with build in bubble trap, a pressure gauge and spindle pump for perfusion pressure adjustment. The perfusion system is connected to a thermostatic circulator which heats the gassed (95% O_2, 5% CO_2) perfusion buffer to 37 °C. In addition, perfusion pressure is recorded continuously and transduced by the pressure recording transducer and visualized and saved on a PC with suitable software. Please click here to view a larger version of this figure.
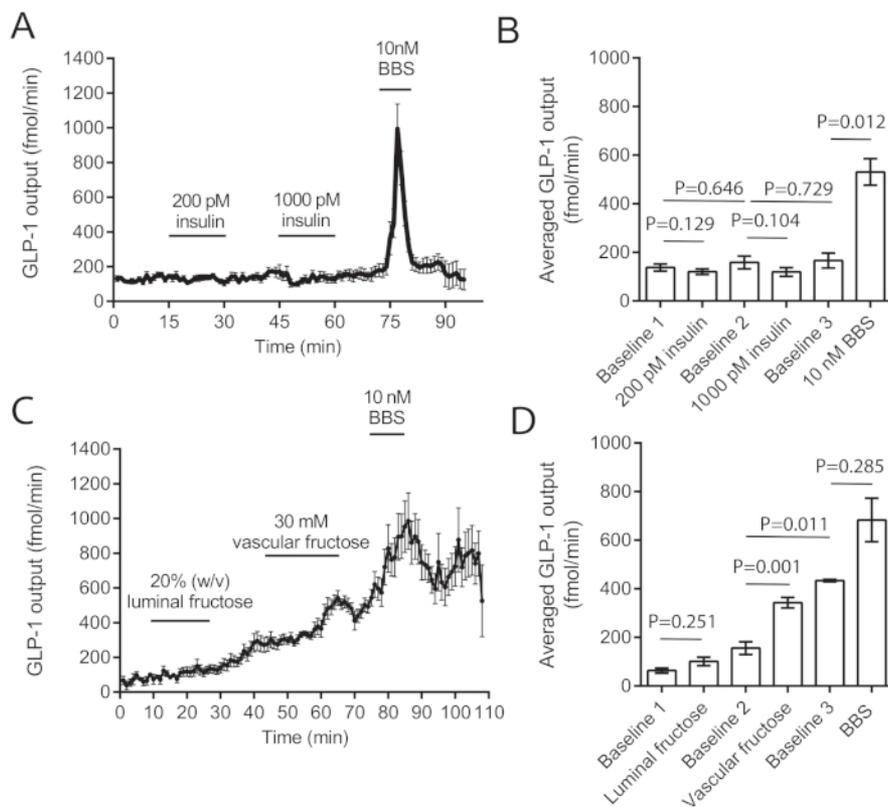

Figure 2: Data from the isolated perfused rat small intestine. (**A**, **B**) an example of a well-performed experiments and reliable data, (**C**, **D**) an example of sub-optimal data where perfusion pressure increased, and perfusion output decreased dramatically over the time course of the study. (**A**) GLP-1 (total) output (fmol/min) is shown at basal conditions and in response to intra-arterial insulin administration (200 and 1,000 pM, as indicated). Bombesin (BBS), a well-known, potent GLP-1 secretagogue, was infused intra-arterially at the end of the experiment to control for responsiveness (pos. control). Data are presented as means ± SEM, n = 4. Please click here to view a larger version of this figure.

Discussion

The isolated perfused rat small intestine is a powerful research tool that allows the dynamics and molecular mechanisms underlying gut hormone secretion to be studied in detail. The most critical step for the successful production of data with this model is the surgical operation. Handling of the gut will inevitably cause some damage to the intestine and should therefore be kept to an absolute minimum. Even more importantly, the speed of operation is key, particularly with regard to the time for the catheter placement in the mesenteric artery. Our experience is that the catheter should be successfully placed, and the perfusion should be initiated within a couple of min after having cut the hole in the vessel. The placement of the catheters is the most challenging part of the operation and is complicated by the small size of the mesenteric artery and the thin wall of the portal vein, and this step requires some practice. Quick insertion of the venous catheter is, however, not as urgent as the case for the mesenteric artery, as the gut is now being perfused and kept alive. The next critical step is to perform the experiment. If the operation was successfully performed, the perfused gut will usually thrive and be responsive for up to 3 h, and perfusion pressure and output will typically remain constant over the entire experiment. The most important factor for a successful experiment at this stage is to avoid bubbles getting into the system, since this causes air embolization which either instantly reduces or eliminates the perfusion output. As the perfusion buffer contains BSA and is gassed to ensure sufficient oxygen delivery to the perfused gut, it is difficult to prevent the formation of bubbles entirely. Bubbles should, however, be trapped in the systems' bubble trap, but this gradually empties over time (the more bubbles, the faster emptying) and it is therefore important to keep a close watch on the bubble trap and refill it with perfusion buffer when needed. It is also important to keep the perfusate pH below 7.5 — at increasing pH, calcium carbonate precipitates may form, obstructing capillary flow and causing a serious rise in perfusion pressure.

The perfused intestine is a rather robust preparation, but it does not tolerate high concentrations of detergents such as bile acids, ethanol and DMSO. Therefore, the use of detergents should preferably be avoided but is sometimes needed to get poorly soluble compounds into solution. The perfused gut generally tolerates detergents administered intra-luminally better than intra-vascularly. For instance, administration of 1 mM taurodeoxycholic acid (a secondary, conjugated bile acid) was tolerated well when infused intraluminally but instantly resulted in blocked perfusion output when delivered intra-arterially[2]. For vascular stimulations, keep the final DMSO concentration below 1% to avoid unspecific secretory responses (DMSO stimulates, for instance, GLP-1 secretion) and to avoid causing tissue damage resulting in increased perfusion pressure and decreased perfusion output. Experiments should be disregarded if the perfusion output decreases by more than 20% over the course of the study, if the perfusion pressure increases more than 20% or if edema develops. Adherence to these macroscopic success criteria (including quick insertion of the catheter in the mesenteric artery) usually results in good quality in ~14/15 experiments.

After completing the experiment, the next critical procedure is to choose the appropriate method and assay for quantification of the hormone(s) of interest. High through-put assaying of peptide hormones can be carried out using immunoassays: radioimmunoassay or ELISA. At any rate, it is highly recommended to validate the respective assays before using them for sample quantification, as not all commercially available assays perform satisfactorily with respect to sensitivity, specificity and accuracy (for examples regarding GLP-1[31]). With respect to specificity, cross-reactivity is, as always, a concern. Unspecific interference and matrix effects are generally less pronounced with artificial perfusates compared to plasma. With regards to sensitivity, it is often much easier to obtain reliable data from perfusions than from *in vivo* studies as the peptide concentrations often are higher because the perfusion effluent is not diluted into the systemic circulation and because elimination processes (by the liver/lung/kidney) are avoided. Typically, baseline concentrations in venous effluents lie within the lower working range of most assays (in case of GLP-1 (total), neurotensin (total) and PYY (total): 8-15 pmol/L) while stimulated responses can reach as high as 200-300 pmol/L[3].

In comparison, baseline values of the same hormones in plasma from healthy humans, rat or mice are typically and 5-10 pmol/L whereas response values are 15-30 pmol/L[3,32].

If the predefined success criteria are adhered to, the generated perfusion data are usually of good quality and the statistical analysis is then straightforward. Compared to secretion of glucagon, insulin and somatostatin from the isolated perfused rat or mouse pancreas, the basal secretion of gut hormones is steady, and rather similar between experiments regarding measured concentrations, and the secretion is not clearly pulsatile. Collectively, the variation in the data set is therefore minimal and a sample size as low as three perfusion experiments is often sufficient to reach statistical significance. However, to standardize and avoid type-2-errors, as well as to reduce the possibility of overlooking less well-pronounced responses, a sample size of six to eight is recommended.

In summary, the isolated perfused rat small intestine is an important, physiologically relevant, experimental model that can be used to investigate the direct effects of a given substance on gut hormone secretion. Important fundamental questions, such as where in the gut a secretagogue stimulates secretion, whether it stimulates secretion through activation of luminally or basolaterally located sensors, and which molecular sensors are activated and thus responsible for the secretion may be addressed in detail with this model. It should, however, be recognized that the uncoupling of the gut from the donor animal may, for some study questions, be of relevance. For instance, the gut interacts closely with the liver (the gut-liver axis) and the brain (the gut-brain axis), and mechanisms that rely on this cross-talk therefore cannot be investigated with this model. The quality of the operation is the critical factor for data quality and the most important factors for a successful operation are to keep handling of the gut to a minimum and to quickly start perfusion of the gut once the mesenteric artery has been cut. It is our experience that it takes 2-4 months of practice before data of good quality are produced. However, once the technique has been learned, the possibilities of this method are almost endless and only limited by the solubility of the test substances and any potential damaging effects test substances may have on the perfused tissue.

Disclosures

The authors of this work declare no potential conflicts of interest relevant to this article.

Acknowledgments

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