The Isolated Perfused Rat Kidney: A Technical Update

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Abstract: The isolated perfused kidney is commonly utilized as a screening tool for renal clearance and metabolism, and to correlate renal drug deposition to renal function. Here, we report on several aspects of this procedure that will facilitate a higher experimental success rate and lead to a reduction in animal use. First, we investigated the utility of inulin and creatinine as commonly used markers to measure glomerular filtration rate. For inulin, in the presence of either 20 mM glucose or 4.5% dextran in the buffer, significant interference was observed using an anthrone-based colorimetric assay. These findings suggest that caution needs to be exercised when using glucose or dextran and when inulin is quantitated using this method. Under these circumstances the use of alternative methods of inulin quantitation such as fluorescently tagged inulin is preferred. Second, we optimized bovine serum albumin (BSA) and BSA/dextran compositions that are routinely recommended as oncotic agents in the perfusion buffer and found that a 4% BSA/1.67% dextran composition had the best viability of kidney biomarkers in accordance with recommended threshold parameters. These considerations will be of particular relevance to researchers utilizing the isolated perfused kidney as a screening tool to measure renal biology and drug metabolism as well as applications to investigate diabetic nephropathy. Key words: diabetic nephropathy, isolated perfused kidney

Introduction

The kidney is a major organ for drug metabolism and metabolite regulation in the body. While an investigation of renal function is essential to understanding metabolite handling and clearance, studies conducted in whole animals or human subjects are often complicated by the metabolic influence of extrarenal tissues and other biochemical factors. Traditionally, the isolated perfused kidney (IPK) has offered an informative system for investigating renal biology without the interference from other organs. The IPK was first established in 1876 by Bunge and Schmeideberg and is commonly utilized as a screening tool for renal clearance and metabolism and to correlate renal drug deposition to renal function [6]. The functional integrity of the entire nephron is preserved, enabling the simultaneous determination of filtration, urinary excretion, and reabsorption, the three major parameters of renal function. The IPK is therefore a useful tool to investigate the biological handling of compounds that would otherwise be difficult to perform in vitro.

The principle of the IPK is to excise the intact kidney whilst aiming to preserve its normal physiological functions after replacing blood with an oxygenated synthetic media. Typical media consists of Krebs-Henseleit bicarbonate (KHB) buffer, which provides the necessary electrolytes. Glucose, amino acids, and bovine serum albumin (BSA) are often included for preservation of
renal function [2] and to provide the necessary oncotic balance in the isolated kidney [3]. While perfusate compositions are generally standardized across studies utilizing the iPK, there are nevertheless variations in reported BSA concentrations ranging from 0.65 to 6% (w/v) [2, 5, 9].

In order to interpret experimental findings using the iPK, it is important that the kidney is viable over the time course of the perfusion. Traditionally, viability has been assessed through several key parameters that must reach minimum acceptable values. Failure to do so results in a rejected experiment and therefore an unnecessary use of an animal. In the present study, we report on several technical factors involving the iPK that impinge on kidney viability or its measurement thereof. Consideration of these factors by researchers using this technique should lead to a higher rate of successful perfusion experiments and a subsequent reduction in animal use.

Materials and Methods

All animal experiments were approved by the University of Auckland’s Animal Ethics Committee. All animals recruited in the studies were housed in pairs and maintained in a controlled environment with a 12 h light/dark cycle and a temperature of 20 ± 2°C. Rats were maintained with free access to tap water and standard rodent chow. For easy identification of rats that were housed in pairs and to ensure that data collection was always correctly assigned to the right animal, a uniquely numbered veterinary electronic tag (Allflex USA, Inc., Dallas, TX, USA) was inserted under the skin at the back of the neck of each rat with a surgical grade single-use 16-gauge needle.

Male Wistar rats (11–12 weeks old) were anaesthetized with 4% isoflurane in 2 l/min oxygen and maintained on 2.5% isoflurane in 2 l/min oxygen. A midline incision was made from bladder to sternum. Major anatomical features were identified, namely the right kidney, right renal artery and vein, aorta, superior mesenteric artery (SMA), and right ureter. These were then cleared of overlying connective tissues and fat. The right ureter was then cannulated with an 8-cm-long PE-10 tubing. Three loose ligatures were made around the right renal artery and proximal and distal mesenteric artery, respectively. Prior to insertion of an arterial cannula, the distal ligature on the SMA was first tied. A small incision was then made proximal to the tied distal ligature on the SMA, and a cannula was inserted into the artery. The cannula was threaded across the aorta and entered the right renal artery. Both the proximal ligature on the superior mesenteric artery and ligature on the renal artery were then tied to secure the cannula in place. The right kidney and its associated vessels and ureter were then rapidly excised en bloc. Only the kidneys that were able to be removed and had ex vivo perfusion commenced within 30 min from the start of the laparotomy were used in these studies. The isolated kidney was then perfused at 36–38°C on a custom-made perfusion apparatus, which comprised a buffer reservoir, peristaltic pump, and a custom-made oxygenator. The latter comprised a pressurized carbogen (95% oxygen, 5% carbon dioxide)-filled cylinder with 15 m of coiled silastic tubing through which buffer traversed as it was oxygenated. Partial pressure of oxygen in the final output buffer was measured at >400 mmHg when the perfusion pump was used at the flow rate of 40 ml/min.

Unless otherwise stated, chemicals were obtained from Sigma Aldrich, BDH chemicals, or Merck at analytical grade or higher. The perfusate comprised 1×KHB buffer (pH 7.4), either fraction V BSA (Gibco/Life Technologies, Melbourne, Australia) or combinations of BSA and dextran (analytical grade, MW 64,000 to 76,000, Sigma-Aldrich, St. Louis, MO, USA), glucose (5 mM), creatinine (200 mg/l), and a 13 mM amino acid solution [2]. Kidney functionality was assessed using criteria including glomerular filtration rate (GFR), glucose reabsorption, Na+ reabsorption, perfusion flow rate, urinary flow rate, and perfusion pressure. GFR was estimated from the clearance of creatinine using the following equation and with the creatinine concentration initially determined by a creatinine assay kit (Cayman, Ann Arbor, MI, USA):

\[
\text{GFR} = \frac{U_{\text{Cr}} \times \text{UFR}}{P_{\text{Cr}}}. 
\]

\(U_{\text{Cr}}\) is the urinary creatinine concentration (mg/ml), \(\text{UFR}\) is the urinary flow rate (ml/min), and \(P_{\text{Cr}}\) is the concentration of creatinine in the perfusate (mg/ml). The percentage fractional reabsorption of glucose and Na+ was calculated using the following equation:

\[
\text{FR}_{(x)} = 1 - \frac{\text{UFR} \times U_{(x)}}{\text{GFR} \times P_{(x)}}, 
\]

where \(\text{FR}_{(x)}\) is the fractional reabsorption of glucose and Na+, \(U_{(x)}\) is the concentration of glucose and Na+ in the
urine, and \( P(x) \) is the concentration of glucose and Na\(^+\) in the perfusate. Glucose concentration was determined using an YSI 2300 STAT plus Glucose and Lactose Analyzer (YSI Inc., Yellow Springs, OH, USA), while Na\(^+\) concentration was measured using a Rapidlab 865 Blood Gas Analyzer (Bayer, Leverkusen, Germany).

**Results and Discussion**

Inulin and creatinine are commonly utilized biomarkers for the calculation of GFR, as both are freely filtered by the glomeruli but not reabsorbed in the nephron. Of the two, inulin is increasingly the preferred marker, as some limitations with creatinine have been reported [8]. Traditionally, the inulin colorimetric assay utilizes an anthrone reagent that reacts with inulin to produce a colored product [5]. In the present study, we identified two factors that could potentially interfere with the derivation of GFR quantitation using the anthrone assay to quantitate inulin. The first factor was the presence of high glucose (20 mM), which is relevant for studies investigating diabetic nephropathy. We found that 20 mM glucose produced significant interference with the anthrone assay (Fig. 1A), however, this interference could be attenuated by pretreatment with glucose oxidase prior to the addition of anthrone reagent [5].

The second factor was the presence of 4.5% dextran (Fig. 1A), which is commonly used in combination with BSA as an oncotic agent because of the relatively high expense of the latter, especially for single-passage experiments. Various BSA or BSA/dextran compositions in the perfusate have been reported with BSA and dextran concentrations ranging from between 0.6 to 6% and 0 to 3.6% respectively [2, 7, 9]. Preincubation of the dextran and inulin solution with ethanol for 16 h, which has been reported to remove dextran from solution [1], partially attenuated the interference. By contrast, no interference by either glucose or dextran was observed using a colorimetric assay for creatinine (Fig. 1B).

These findings suggest that the presence of either high glucose or dextran can potentially interfere with inulin quantification using the anthrone method. In both cases, the increased absorbance would lead to an underestimation of GFR. Consequently, these findings support the use of alternative methods for inulin quantitation such as fluorescently tagged inulin [4].

We also compared three different BSA/dextran compositions that are routinely employed in other IPK studies in order to optimize the experimental protocol utilizing the single-passage set-up and using creatinine as the marker (Fig. 2). We confined these studies to a pump-driven experimental setup in which all standard viability criteria were satisfied [6], and it was clearly superior to a gravity-driven system (Table 1). Here, the flow rate was adjusted in order to keep the arterial pressure within the range of 90 to 110 mmHg.

The average perfusion pressure (Fig. 2A) and venous flow rate (Fig. 2B) did not differ significantly between the three different perfusate compositions and was consistently maintained around 100 mmHg and 30 ml/min respectively throughout the time course of the experiments. However, kidneys perfused by the buffer containing only 6% BSA displayed a significantly lower urinary flow rate (Fig. 2C) and GFR (Fig. 2D) throughout the 60-min period as compared with the other two BSA/dextran compositions, comprising 4% BSA/1.67% dextran, and 0.65% BSA/3.6% dextran respectively. By contrast, perfusates containing 0.65% BSA/3.6% dextran displayed significantly lower glucose reabsorption (Fig. 2E) and tubular reabsorption (Fig. 2F). We did not check levels of dextran in the urine. However, due to the high molecular weight of the dextran (MW 64,000 to 76,000), it is unlikely that any leakage occurred.
Fig. 2. Effects of perfusate composition on kidney functional parameters using a pump-driven perfusion system. Isolated kidneys were perfused with buffer containing either 6% BSA (●), 4% BSA/1.67% dextran ( ■ ), or 0.65% BSA/3.6% dextran (◆). Following a stabilization period of 30 min, measurements were sampled from pooled fractions at 10-min intervals. Data represent means ± SEM with n=3 kidneys for each group. Statistical analysis was performed from analyses on the area under the curve using one-way ANOVA followed by Tukey’s multiple comparison test. (A) Arterial pressure. (B) Venous flow rate. (C) Urinary flow rate: 6% BSA vs 4% BSA/1.67% dextran (P<0.01); 6% BSA vs 0.65% BSA/3.6% dextran (P<0.001). (D) GFR: 6% BSA vs 4% BSA/1.67% dextran (P<0.001); 6% BSA vs 0.65% BSA/3.6% dextran (P<0.001). (E) Fractional reabsorption of glucose: 0.65% BSA/3.6% dextran vs 4% BSA/1.67% dextran (P<0.01); 0.65% BSA/3.6% dextran vs 6% BSA (P<0.001). (F) Fractional reabsorption of Na+: 0.65% BSA/3.6% dextran vs 4% BSA/1.67% dextran (P<0.01); 0.65% BSA/3.6% dextran vs 6% BSA (P<0.001).
In summary, we identified two experimental factors, 20 mM glucose and 4.5% dextran, that interfered with the anthrone-based method for inulin quantitation and would lead to an underestimation of GFR. The interference with glucose was completely attenuated by treatment with glucose oxidase, while the interference due to dextran was only partially attenuated through ethanol treatment. Of the perfusates investigated, only the 4% BSA/1.67% dextran combination preserved renal tubular function, adequate urine flow rate, and GFR in accordance with recommended threshold parameters [6]. Our data suggests that caution needs to be exercised when using either high glucose or dextran in combination with inulin as a marker for GFR. Under these circumstances, the use of alternative methods of inulin quantitation such as fluorescently tagged inulin is preferable [4]. These considerations will be of particular relevance to researchers utilizing the isolated perfused kidney as a screening tool to measure renal clearance, and drug metabolism as well as investigations into diabetic nephropathy.

### Acknowledgments

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### Table 1. Comparison of kidney viability parameters between gravity feed and pump perfusion systems

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<tr>
<td>Arterial perfusion pressure (mmHg)</td>
<td>90–110</td>
<td>100</td>
<td>99 ± 4.9</td>
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<tr>
<td>Urine flow rate (µl/min)</td>
<td>&gt;30</td>
<td>11 ± 1.46</td>
<td>89 ± 5.78</td>
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<tr>
<td>Perfusion flow rate (ml/min)</td>
<td>15–40</td>
<td>14.5 ± 2.1</td>
<td>31 ± 2.7</td>
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<td>Fractional glucose reabsorption (%)</td>
<td>&gt;90</td>
<td>98 ± 3.6</td>
<td>96 ± 3.8</td>
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<tr>
<td>Fractional Na+ reabsorption (%)</td>
<td>&gt;85</td>
<td>90 ± 2.3</td>
<td>89 ± 2.5</td>
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<td>GFR (ml/min)</td>
<td>&gt;0.5</td>
<td>0.13 ± 0.03</td>
<td>0.69 ± 0.04</td>
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Isolated kidneys were stabilized for 30 min upon initiation of perfusion which comprised 50 μM MI, 5 μM DCI, 5 mM glucose, 200 mg/l creatinine, 4% (w/v) BSA, 1.67% dextran, 13 mM amino acids, and 1 × KHB. Kidney viability parameters were then monitored for 20 min (n = 5 kidneys for each system). Data represent means ± SEM. Minimum acceptable values are based on Taft, 2004 [6].

### References


