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Review

THE ISOLATED PERFUSED KIDNEY MODELS - CERTAIN ASPECTS

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ABSTRACT

This review is an attempt for a brief tracing of the main moments in the development of isolated perfused kidney method. It is emphasized the significance of this model and its application in various fields of science. The versatility of the isolated perfused kidney method is useful for modulating a number of pathophysiological processes, which could be helpful in studying and improving a better treatment. Isolated perfused kidney could be beneficial in the establishment of the preservation techniques used in kidney transplantation.The opportunities for the surgical procedures, the study design, the solutions for perfusion and the possible modifications of the method are described. The purpose of this paper is to regain the value of the isolated perfused kidney. This model, although expensive and sophisticated, is still valid today, and is worth the effort to be used.

Key words: isolated kidney, experimental models, preparations, perfusion

The study of the isolated perfused kidney (IPK) began in the early decades of the 20th century (1, 2) enabling the development of renal physiology, pathophysiology and pharmacology. The IPK is an experimental model that can be used to investigate the glomerular filtration rate (GFR), the tubular reabsorbtion of water and sodium and their urine excretion, some aspects of the secretory function of the kidney (3), the renal metabolism, as well as other functions, like the renal reperfusion and nonlinear drug excretion (4, 5), the effects of metabolites, drugs, hazardous substances on renal functions (6) and the renal resistance to such influences (7). The model of IPK allows control of the perfusion pressure, renal flow, and concentrations of the substances in the system.

Subsequently, the scope of the model has broadened to include studies of the dynamic autoregulation in the kidney (8), to make non-filtering kidney (NFK) preparations for investigating tubular epithelial cells metabolism (9), to induce and explore the diabetic kidney (10), to observe changes in the afferent and efferent arterioles, provoked by different substances (11, 12).

Over the last decade IPK has become a convenient model for developing new strategies to improve kidney function after renal transplantation (4) and could be useful in the establishing of preservation techniques for kidneys have taken from donors (4).

The first experimental animals were dogs (2, 4, 13) and cats (1). One of the reasons for using larger animals had been the need for sufficient material for analysis. The modern microanalytic methods have greatly scaled down this requirement (3).

On the next stage of development of the IPK technique, the most common species is the rat (3, 5, 7-12, 14-19). Rat kidneys are preferred due to their small size and the fact that they offer no special difficulties of the operative technique. The perfused rat kidney may in

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future conveniently replace for many purposes the use of larger animals (3). To reduce the sophistication and cost of the technique as well as to introduce and test newer substances, mice are starting being used (20, 21). Other researchers have an opinion that physiological and morphological parameters of small laboratory animal kidneys are difficult to compare to human renal parameters (6) and prefer working on pigs (1, 6, 13, 19).

At the beginning of the surgical procedure, the test animal is anesthetized. Some authors prefer inhalation anesthesia with isoflurane (20, 22), while others - intraperitoneal injection of barbiturates (23, 24). Mechanical ventilation via tracheotomy is needed to extend the vitality of the tissues and to prevent respiratory failure (25, 26). The abdominal cavity is usually opened by a medial line incision. The animal is eviscerated and the intestines are displaced out. The abdominal aorta, renal arteries, superior mesenteric artery and inferior vena cava are exempted from surrounding connective tissue. An important point is the imposition of prior non-tight ligatures around the vessels. This is for fixing catheters in the vessels and limiting blood loss.

Most researchers work on the right kidney, because the mesenteric artery arises from the aorta at the same level as the right renal artery and the catheter can be passed from one to the other without blood loss and without stopping the blood flow to the kidney (3). Another method for cateheterisation is by aorta abdominalis. The aorta is clamped above the point of separation of the renal artery. Under microscope control (for mice and rat kidney) the aorta abdominalis is incised with a cut that goes in direction 45 degrees and covers 2/3 of the lumen of the vessel. A catheter is inserted through the opening in direction retrograde to the blood flow. Some authors prefer to fix the catheter in the aorta abdominalis (27), while others move it 2-3 mm up into a. renalis (10). The pre-imposed ligature is tightened, while starting the perfusion of the kidney. The catheter in the venous vessel (v. renalis or v. cava inferior), might be connected to the circulating system or may end opened for taking blood samples. The left kidney usually is used as a reference weight (14).

There are two main categories of methods for perfused kidney: *in vitro* perfusion – the kidney is detached carefully of the body and placed in a heated chamber, and *in situ* perfusion – the kidney remains in the body of the experimental animal (28). When the kidney is isolated, it is put in a heated chamber (23, 24), or placed in a solution, containing all necessary substances for kidney metabolism (9). There are authors, who succeed to isolate and perfuse both kidneys (4, 28). In this experimental model the clamp is placed on the aorta abdominalis between the two a. renalis, the right kidney is catheterized by the described method, while the catheterization of the left one is through aorta thoracica. It is possible both kidneys to be perfused with different solutions.

Determining the vitality of the kidney during perfusion can be done by using different criteria: the changes in the perfusion pressure, the renal perfusion flow, GFR, the electrolyte and glucose reabsorption, the oxygen consumption and urine concentration (4, 8). Other authors use measurements of glucose and creatinine in the urine (3, 9, 15, 19). GFR can be estimated by clearance of creatinine or inulin. Inulin is a preferred marker for determination of GFR in IPK experiments. Although it is used often as a radioactive marker (14 C-inulin) in the literature are presented and non-radioactive methods (29, 30). Perfusion pressure is kept within 110-120 mmHg, by adjustment of the perfusion rate (25-40 ml/min), which is important for maintaining the GFR. The experiment is terminated if any criterion for vitality is out of range, for example: the perfusion pressure above 120 mmHg, the renal perfusion flow below 20 ml/min (28) or fall of kidney reabsorption below 93% (28).

All methods described above, are carried out at physiological temperature. If the temperature of the perfusion solution is kept at 8°C, the tubular processes are excluded, which reduces energy costs while the glomerular filtration is maintained (21).

Once the kidney is isolated, it must be maintained vital by being perfused with a solution, pumped with a certain pressure. Maximum similarity of the perfusion solution to blood is desirable, in order to fully simulate the natural environment of the kidney *in vivo*. The first experiment with kidney perfusion was with the saline of Krebs & Henseleit in 1932 (1, 2, 4, 14). This medium contains electrolytes, glucose, oncotic agents, and some

other substances like inulin, amino acids, etc. Glucose is added to the perfusion solution as a metabolic substrate, providing the energy needs of tubular cells. The reabsorption of glucose is one of the criteria for kidney vitality, particularly for the function of the proximal renal tubules. The presence of oncotic agent is extremely important because it creates a colloid-osmotic pressure necessary for achieving normal glomerular filtration, reabsorption of water and electrolytes in the proximal renal tubules. As an oncotic agent usually is used bovine serum albumin (BSA) in different concentrations (31). The amount of protein depends on the experimental method and varies between 5% and 7.5% (32). Albumin is employed in concentration such that the colloid osmotic pressure of the perfusion solution approaches the physiological values (3, 17) and the result is an increase in the survival of the isolated kidney (17). However, BSA is a costly substance, and this requires the application of alternative methods, like BSA / dextran in different proportions (33). Perfusion solutions containing dextran and albumin had similar efficiency in the temperature range of 8 °C (28). IPK experiments conducted with BSA plus dextran and those conducted only with BSA showed that in kidneys perfused with BSA and dextran GFR was higher and its level remained stable over throughout the experimental period of 130 minutes compared to those, perfused only with BSA. (33). Other substances, which have similar properties to albumin, such as orosmucoid are tested (34).

Tissue hypoxia should be taken into account in longer experiments. In those cases either direct oxygenation of the perfusion solution is employed (34) or the same effect is achieved by adding erythrocytes up to hematocrit of 0.33 (24).

There are experimental models based on perfusion without red cells (7). These are models of perfusion with dextran (28), with Ficoll (27) and with Tyrode solution (4). The vitality of the kidney in such cases is reduced to 30 min which is sufficient for experiments studying excretion of substances (10, 35). In order to prolong the duration of experiment and to obtain an effective function of IPK, additional substances, such as amino acids, buffers, etc. are added to the perfusion solution (7, 9, 17). They are not utilized by the kidney but are involved in co-transport mechanisms (29). Adding amino acids to the perfusion solution has a protective effect on tubular cells and improves the vitality of the kidney. In several studies of the effect of amino acids on the function of the IPK is demonstrated that L-glycine and L-alanine at physiological concentrations, have a protective effect on tubular epithelial cells. (36). Glutamate, cysteine + glutamate and L-serine all have little or no protective effect against serious damage of tubular cells (37).

Another important stage in the development of IPK, is the inclusion of the dialysis. When a dialyzer was included in the perfusion circuit, glomerular filtration rate and sodium transport remained nearly constant for 2 h, which decreased glucose formation in the absence of added precursors (3, 17). In normal dialysis substances are withdrawn from circulation, but in the IPK experiments dialysis is used to enrich continuously circulating solution and allows reperfusion of the main costly substances. Further improvement in recent years are studies with electricity that investigated the activity of the vascular wall of kidney vessels (18).

For the purpose of studying separately the glomerular and tubular function, some experimental models break the connection between the tubules and cortex employing different methods, for example hydronephrosis (8, 11). Studies involving microscopy provide direct visualization of the microvascular system of the kidney (8, 11). The NFK preparation is normally used for assessment of drug transport into renal tubular cells (9). In this preparation, the glomerular filtration is stopped by the ureter ligation and by the high oncotic pressure, exerted by 8% albumin in perfusion solution. Hence, a studied drug could only able to gain access to the renal tubule indirectly by means of the post glomerular circulation (9). Other authors use the model of the fixed kidney. This is a modification of the IPK model, developed for the study of charge related characteristics of glomerular capillary wall permeability and selectivity. The tubular transport processes in this model are completely blocked by a perfusion fixation with glutaraldehyde and the glomerular permeability properties could be observed directly. The collected urine represents the glomerular ultrafiltrate and the fixed kidney can thus be considered as a membrane (7, 14). In some experimental methods only parts of

the kidney are perfused using micro puncture of single nephrons, isolated glomeruli and isolated glomerular or endothelial cells. The micro puncture of isolated single nephrons examines pressure, flow and transport of low molecular weight substances (38 - 40). Isolated glomeruli are used in the study of the basal membrane: its aging (41), its ionic charges (42) and transport processes through it (43, 44). Isolated cells are used in the study of the role of proteoglycans for nephrotic syndrome (45 - 47).

It must be taken into account that the response of the isolated kidney to the effects of reagents is different from the one *in situ* due to the suppressed humoral regulation and the absence of innervation. The studies of these differences are extremely important for improving the methods of renal transplantation. In IPK models, the kidney is analogous up to some extent to that taken form the donor. Some experiments can be performed *in vivo*, but are readily achievable with IPK, for example studying the importance of the electric charges for the membrane permeability, the factors leading to development of nephrotic syndrome, the tubular reabsorption, the affinity of immunoglobulin complexes to the glomerular membrane and others. The study of the kidney endocrine function, such as the secretion of renin, is much more accurate for models with a whole organ, rather than slices.

In conclusion, the model of IPK remains actual today (6, 20, 22 - 24, 35, 46). It is applicable for testing the kidney functions, for modeling different pathophysiological processes, for solving many problems associated with renal transplantation and for preclinical studying of various pharmaceutical products.

REFERENCES

1. Macgregor, R. and Peat, S., The histamine-histaminase system in the isolated perfused kidney-lung preparation. *J Physiol*, 77(3): 310–318, 1933.
2. Hemingway, A. and Schweitzer, A., The excretion of diodone by the isolated perfused dog kidney. *J Physiol*, 102(4):491-5, 1944.
3. Nishiitsutsuji-Uwo, J., Ross, B., Krebs, H., Metabolic activities of the isolated perfused rat kidney. *Biochem J*, 103(3):852-62, 1967.
4. Höchel, J., Lehmann, D., Fehrenberg, C., Unger, V., Groneberg, D., Grosse-Siestrup, C., Effects of different perfusates on functional parameters of isolated perfused dog kidneys. *Nephrol Dial Transplant*, 18(9):1748-54, 2003.
5. Aiba, T., Kubota, S., Koizumi, T., Application of a non-linear dispersion model to analysis of the renal handling of p-aminohippurate in isolated perfused rat kidney. *Biol Pharm Bull*, 22(6):633-41, 1999.
6. Unger, V., Grosse-Siestrup, C., Fehrenberg, C., Fischer, A., Meissler, M,, Groneberg, D.A., Reference values and physiological characterization of a specific isolated pig kidney perfusion model. *J Occup Med Toxicol*, 29(2):1, 2007.
7. Ciarimboli, G., Hjalmarsson, C., Bökenkamp, A., Schurek, H., Haraldsson, B., Dynamic alterations of glomerular charge density in fixed rat kidneys suggest involvement of endothelial cell coat. *Am J Physiol Renal Physiol*, 285(4):F722-30, 2003.
8. Cupples, W. and Loutzenhiser, R., Dynamic autoregulation in the in vitro perfused hydronephrotic rat kidney. *Am J Physiol*, 275(1 Pt 2):F126-30, 1998.
9. Sirianni, G., Pang, K., Intracellular and not intraluminal esterolysis of enalapril in kidney. Studies with the single pass perfused nonfiltering rat kidney. *Drug Metab Dispos*, 26(4):324-31, 1998.
10. Weiss, C., Passow, H., Rothstein, A., Autoregulation of flow in isolated rat kidney in the absence of red cells. *Am J Physiol*, 196(5):1115-8, 1959.
11. Epstein, M., Calcium antagonists and renal hemodynamics: implications for renal protection. *J Am Soc Nephrol*, 2(2):S30-6, 1991.
12. Quilley, C., Lin, Y., McGiff, J., Chloride anion concentration as a determinant of renal vascular responsiveness to vasoconstrictor agents. *Br J Pharmacol*, 108(1):106-10, 1993.
13. Wilcox, C., Regulation of renal blood flow by plasma chloride. *J Clin Invest*, 71(3):726-35,1983.
14. Ciarimboli, G., Schurek, H., Zeh, M., Flohr, H., Bökenkamp, A., Fels, L., Kilian, I., Stolte, H., Role of albumin and glomerular capillary wall charge distribution on glomerular permselectivity: studies on the perfused-fixed rat kidney model. *Pflugers Arch*, 438(6):883-91, 1999.

15. Radermacher, J., Klanke, B., Schurek, H., Stolte, H., Frölich, J., Importance of NO/EDRF for glomerular and tubular function: studies in the isolated perfused rat kidney. *Kidney Int*, 41(6):1549-59, 1992.
16. Frölich, J., Yoshizawa, M., Renal vascular effects of leukotriene C4 in the isolated perfused kidney of the rat. *Br J Pharmacol*, 92(2):311-8, 1987.
17. Schurek, H. and Alt, J., Effect of albumin on the function of perfused rat kidney. *Am J Physiol Renal Physiol,* 240: F569-F576, 1981;
18. Collis, M. and Vanhoutte, P., Vascular reactivity of isolated perfused kidneys from male and female spontaneously hypertensive rats. *Circ Res*, 41(6):759-67, 1977.
19. Klanke, B., Simon, M., Röckl, W., Weich, H., Stolte, H., Gröne, H., Effects of vascular endothelial growth factor (VEGF)/vascular permeability factor (VPF) on haemodynamics and permselectivity of the isolated perfused rat kidney. *Nephrol Dial Transplant*, 13(4):875-85, 198.
20. Jeansson, M., Granqvist, A., Nyström, J., Haraldsson, B., Functional and molecular alterations of the glomerular barrier in long-term diabetes in mice. *Diabetologia*, 49(9):2200-9, 2006.
21. Jeansson, M. and Haraldsson, B., Glomerular size and charge selectivity in the mouse after exposure to glucosaminoglycan-degrading enzymes. *J Am Soc Nephrol*, 14(7):1756-65, 2003.
22. Pollock, D., Endothelin, Angiotensin, and Oxidative Stress in Hypertension. *Hypertension*, 45(4):477-80, 2005.
23. Boesen, E. and Pollock, D., Acute increases of renal medullary osmolality stimulate endothelin release from the kidney. *Am J Physiol Renal Physiol*, 292(1):F185-91, 2007.
24. Schneider, M., Inscho, E., Pollock, D., Attenuated vasoconstrictor responses to endothelin in afferent arterioles during a high-salt diet. *Am J Physiol Renal Physiol*, 292(4):F1208-14, 2007.
25. Bakoush, O., Tencer, J., Torffvit, O., Tenstad, O., Skogvall, I., Rippe, B., Increased glomerular albumin permeability in old spontaneously hypertensive rats. *Nephrol Dial Transplant*, 19(7):1724-31, 2004.
26. Hjalmarsson, C., Ohlson, M., Haraldsson, B., Puromycin aminonucleoside damages the glomerular size barrier with minimal effects on charge density. *Am J Physiol Renal Physiol*, 281(3):F503-12, 2001.
27. Ciarimboli, G., Hjalmarsson, C., Bökenkamp, A., Schurek, H.J., Haraldsson, B., Dynamic alterations of glomerular charge density in fixed rat kidneys suggest involvement of endothelial cell coat. *Am J Physiol Renal Physiol*, 285(4):F722-30, 2003
28. Cox, P., Moons, W., Russel, F., van Ginneken, C., Renal handling and effects of S(+)-ibuprofen and R(-)-ibuprofen in the rat isolated perfused kidney. *Br J Pharmacol*, 103(2):1542-6, 1991.
29. White, R.P. and Samson, F.E. Jr**.,** Determination of inulin in plasma and urine by use of anthrone. *J Lab Clin Med*, 43(3):475-8, 1954.
30. Heyrovsky, A., A new method for the determination of inulin in plasma and urine. *Clin Chim Acta*, 1(5):470-4, 1956.
31. Taft, D.R., The isolated perfused rat kidney model: a useful tool for drug discovery and development. *Curr Drug Discov Technol*,1(1):97-111, 2004.
32. Maack, T., Physiological evaluation of the isolated perfused rat kidney. *Am J Physiol*, 238(2):F71-8, 1980.
33. Wang, J., Nation, R.L, Evans, A.M, Cox, S., Isolated rat kidney perfused with dextran and bovine serum albumin: a stable model for investigating renal drug handling. *J Pharmacol Toxicol Methods*, 49(2):105-13, 2004.
34. Haraldsson, B., Johnsson, E., Rippe, B., Glomerular permselectivity is dependent on adequate serum concentrations of orosomucoid. *Kidney Int*, 41(2):310-6, 1992.
35. Venturoli, D. and Rippe, B., Ficoll and dextran vs. globular proteins as probes for testing glomerular permselectivity: effects of molecular size, shape, charge, and deformability. *Am J Physiol Renal Physiol*, 288(4):F605-13, 2005.
36. Baines, A.D., Shaikh, N., Ho, P., Mechanisms of perfused kidney cytoprotection by alanine and glycine. *Am J Physiol*, 259(1 Pt 2):F80-7, 1990.
37. Silva, P., Rosen, S., Spokes, K., Epstein, F.H. Effect of glycine on medullary thick ascending limb injury in perfused kidneys. *Kidney Int*, 39(4):653-8, 1991.

38. Bartoli, E., Romano, G., Favret, G., Segmental reabsorption measured by micropuncture and clearance methods during hypertonic sodium infusion in the rat. *Nephrol Dial Transplant*, 11(10):1996-2003, 1996.
39. Romano, G., Favret, G., Federico, E., Bartoli, E., The validity of the recollection technique in micropuncture experiments on the rat kidney. *Exp Physiol*, 82(1):161-9, 1997.
40. Bartoli, E., Romano, G., Favret, G., Micropuncture and clearance measurements of segmental reabsorption by the rat nephron. *Nephrol Dial Transplant*, 11(2):275-81, 1996.
41. Neumann, K., Kellner, C., Kühn, K., Stolte, H., Schurek, H., Age-dependent thickening of glomerular basement membrane has no major effect on glomerular hydraulic conductivity. *Nephrol Dial Transplant*, 19(4):805-11, 2004
42. Comper, W., Lee, A., Tay, M., Adal, Y., Anionic charge concentration of rat kidney glomeruli and glomerular basement membrane. *Biochem J*, 289 (Pt 3):647-52, 1993.
43. Tay, M., Comper, W., Singh, A., Charge selectivity in kidney ultrafiltration is associated with glomerular uptake of transport probes. *Am J Physiol*, 260(4 Pt 2):F549-54, 1991.
44. Comper, W., Tay, M., Wells, X., Dawes, J., Desulphation of dextran sulphate during kidney ultrafiltration. *Biochem J*, 297(Pt 1): 31–34, 1994.
45. Fishman, J. and Karnovsky, J., Effects of the aminonucleoside of puromycin on glomerular epithelial cells in vitro. *Am J Pathol*, 118(3): 398–407, 1985.
46. Björnson, A., Moses, J., Ingemansson, A., Haraldsson, B., Sörensson, J., Primary human glomerular endothelial cells produce proteoglycans, and puromycin affects their posttranslational modification. *Am J Physiol Renal Physiol*, 288: F748-F756, 2005.
47. Sörensson, J., Björnson, A., Ohlson, M., Ballermann, B., Haraldsson, B., Synthesis of sulfated proteoglycans by bovine glomerular endothelial cells in culture. *Am J Physiol Renal Physiol*, 284: F373-F380, 2003