ILLUMINATIONS

An educational tool to improve understanding of angiotensin II function and the adrenergic system in renal circulation

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INTRODUCTION

Renal physiology is known as a hard subject to be taught. Health sciences students starting to learn renal physiology usually struggle with concepts such as glomerular filtration rate (GFR) and filtration fraction and the adjustment of these parameters by arteriolar vasomotion.

The GFR is the process that, together with tubular processing, starts and determines the main renal function, its excretory action, and, therefore, the maintenance of hydrosaline balance between the liquid compartments of the body, as well as the elimination of metabolic waste. Hydrostatic capillary pressure is the primary biophysical determinant of the GFR, and it is transmitted to the renal circulation from the systemic arterial pressure. Many factors, including angiotensin II (ATII) and the sympathetic nervous system, can modify the diameter of the afferent (AA) and efferent arterioles (EA), changing the capillary hydrostatic pressure and kidney filtration capacity. The comprehension of these key concepts is critical, especially considering that this mechanism is involved in many neurohumoral processes regulating renal blood flow and the GFR.

To contextualize this information for future doctors, nurses, and physiotherapists, it is necessary to explain how the kidneys act on the GFR, in response to hemodynamic changes that should occur in critical clinical situations, such as hypovolemic shock. It is also possible to relate the pharmacological effects of some medicines that act on this system and the clinical consequences resulting from the modification of the renal blood flow and the GFR. Therefore, we propose, through a simple didactic tool reproducible with everyday laboratory materials, to offer a visual and interactive model for the demonstration of simple renal physiology concepts, a subject that students often find challenging.

MATERIALS AND METHODS

This article describes an educational tool we provided to undergraduate students of the University of Caxias do Sul, in Caxias do Sul, Brazil. The constructed apparatus is shown in Fig. 1A.

The following materials were used:
1. One 12-liter semitransparent plastic water carboy
2. A tap
3. One 80-mm-diameter (internal) stainless steel nozzle
4. One 90-mm-diameter (internal) silicone tube (it must be long enough to connect the tap to the carboy)
5. One 90-mm-diameter (internal), 8-cm-long silicone tube
6. One 80-mm-diameter (internal), 15-cm-long silicone tube
7. One 80-mm-diameter (internal), 10-cm-long silicone tube
8. One 60-mm-diameter (internal), 15-cm-long silicone tube
9. One 60-mm-diameter (internal), 10-cm-long silicone tube
10. One 30-mm-diameter (internal), 10-cm-long silicone tube
11. One 90-mm-diameter (internal) Y glass bifurcation
12. Two 500-ml low-form beakers (Griffin beaker)
13. Three claw clamps
14. Red adhesive tape
15. Blue adhesive tape
16. Chronometer
17. Pen or pencil
18. Experimental protocols
19. Scissors or stiletto

Assembly Method

The assembly method is as follows:
1. To prepare the carboy, we made a transverse cut all over its circumference at the open end (which remains open). A hole of the nozzle’s diameter was made 5.5 cm away from the carboy base, and the nozzle was fixed to it. A blue band was attached 7.5 cm above the carboy nozzle, which determines the height at which the water column should be kept during most of the experiment. This band was called the “equilibrium point” (EP). In the same way, below the blue band and 2.5 cm above the nozzle, a red band was attached that represents the “hypovolemia point” (HP).
2. A 90-mm-diameter silicone tube was connected to the tap by one extremity. The other extremity was put at the carboy opened side. This was called “extremity 0” (E0).
3. The 90-mm-diameter and 8-cm-long silicone tube was connected to the nozzle (at the carboy base), making a water system. This was named “extremity 1” (E1).
4. The Y glass bifurcation was connected to the plumbing.
5. The two 15-cm-long silicone tubes (diameters 80 and 60 mm) were connected and successively coupled to one of the extremities of the bifurcation, named “extremity 2” (E2).
6. The three 10-cm silicone tubes (diameters 80, 60, and 30 mm) were united and successively coupled to the other bifurcation extremity, named “extremity 3” (E3).
7. The beakers were put under the extremities E2 and E3 (one beaker for each extremity).
8. A blue mark was made at the tap-valve opening position, at which the E0 outflow was equal to the E2 plus E3 outflow. Therefore, the amount of water entering the tap was the same as that flowing through the plumbing, and the water column remained constant in the EP.
9. The claw clamps were adapted to perform the selective vasoconstriction of the silicone extremities E1 and E2.

Correlating to physiological aspects, the water volume contained in the carboy represented the extracellular fluid volume or, more specifically, the blood plasma volume. Consequently, E0 represented the fluid intake that fulfilled the blood plasma volume. It is possible to observe in Fig. 1B that there were three extremities coupled to the Y bifurcation, which represented the renal glomerulus. E1 represented the AA, E2 represented the EA, and E3 represented the Bowman’s capsule (BC). The flows passing through the extremities AA and BC represented the renal plasma flow (RPF) and the GFR, respectively. From now on, just the anatomohyological terms and acronyms will be used to refer the extremities.

The beakers were used to assess the EA output volume, the RPF, and the glomerular capillary output volume, the GFR. The drainage from the operating apparatus was captured by both extremities EA and BC simultaneously and without any fluid loss. It is important to emphasize that the entire system should be filled with water before emptying, to avoid trapped bubbles. The volumes obtained by the beakers should be measured after 10 s of ideal system operation.

Before the beginning of the experiment, we calibrated the apparatus according to the characteristics of the laboratory tap. We measured the total water flow through the extremities EA and BC for 10 s, with the water level constantly at the carboy blue mark. In this case, we obtained an approximate total volume of 240 ml with the tap we used. We then determined the tap setting for this flow (24 ml/s) and marked the valve with a blue band at this point. Similarly, we made a mark on the tap when we obtained a constant water volume for the HP.

The experiments were conducted by groups of a maximum of five students, oriented, only if needed, by a monitor or teacher. The instructions for the model, the protocols for each test, and the explanations of the physiological concepts were described in the protocols, and each student received a copy at the beginning of the class.

**Lesson Protocol**

The nephron is the kidney’s functional unit and consists of the renal glomerulus and the renal tubules. Inside each glomerulus, there are a number of capillaries that originate from the AA and converge at the EA. The hydrostatic pressure inside the capillary effects the production of the initial ultra-filtrate, and the pressure is determined by the constriction rate of AA and EA. The following hydraulic tool aims to demonstrate how the sympathetic nervous system and ATII interfere with glomerular filtration via arteriolar constriction, as well as the basic biophysical principles involved.

**Experiment 1. Renal plasma flow, glomerular filtration, and filtration fraction.** The fluid volume contained in the carboy represents the extracellular fluid volume, which corresponds to ~60% of an adult’s total body fluid. The amount of fluid that flows from the carboy to the plumbing corresponds to the RPF. In the human body, 25% of blood pumped by the heart, per minute, goes to the kidneys, reaching the renal glomerulus through the AA, which only filters a small portion. The amount of plasma passing through the glomerular capillaries is measured by the GFR. The ratio between the GFR and the RPF is called the filtration fraction (FF), which measures how much of the RPF is filtered.

\[
\text{Filtration Fraction (FF)} = \frac{\text{Glomerular Filtration Rate (GFR)}}{\text{Renal Plasma Flow (RPF)}}
\]

In the model, the three extremities below represent the renal glomerulus. The flows in each segment are described in the subtitle.

To start the experiment, follow the instructions below:
1. Turn the tap on and fill the carboy until the water reaches the blue band.
2. Open the valve to the blue mark to keep the water column level at the carboy’s blue band; this is the model’s EP for body fluids.
3. Make sure there are no air bubbles inside the plumbing and the water is flowing freely.

Fig. 1. Functional diagram. A: schematics of the model, with the local tap and the beakers for volume collection. B: illustration corresponding to the renal glomerulus (for additional details, see text). E0–E3, extremity 0–3; ECFV, extracellular fluid volume.
4. Collect all of the fluid drained by extremities EA and BC in two beakers, simultaneously and without fluid loss, for 10 s.
5. Record the values of the volume/flows in 10 s below:
   Renal plasma flow (the sum of the glomerular filtration rate and the flow through the efferent arteriole): ___________________
   Glomerular filtration rate: _____________________________
   Filtration fraction: _____________________________

   Only one-fifth of the blood that reaches the kidney is filtered; consequently, the ratio between GFR and RPF is ~0.20 (8).

   Experiment 2. Efferent arteriole vasoconstriction and angiotensin II action. The constriction of the AA and EA affects the glomerular filtration parameters in different ways, and there are many factors (e.g., myogenic, hormonal, and neural) regulating constriction. The renin-angiotensin system is one of them, activated when the renal perfusion pressure is reduced, resulting in the generation of both local and systemic peptide ATII. This hormone causes constriction of the AA and EA, but preferably increases the constriction of the EA. This can be explained by the smaller diameter of the EA, and, consequently, constriction on this arteriole produces a greater resistance increase than the AA. Also, the ATII induces nitric oxide and prostaglandin release at the AA, causing vasodilation and minimizing local vasoconstriction. Lastly, the ATII decreases vasoconstriction in the AA through the stimulation of the type II angiotensin receptor, which causes local vasodilatation.

   Experiment 2 aims to simulate the action of ATII on the renal arterioles. Follow the instructions below:
   1. Adjust the claw clamp over the EA tube, without pressing it.
   2. Proceed with six complete rotations of the claw presser.
   3. With the beakers, measure the fluid volume flowing through EA and BC, simultaneously and without fluid loss, for 10 s.
   4. Record the values of the volume/flows in 10 s below:
      Renal plasma flow (the sum of the glomerular filtration rate and the flow through the efferent arteriole): ___________________
      Glomerular filtration rate: _____________________________
      Filtration fraction: _____________________________

   The preferential increase in the EA resistance, induced by ATII, contributes to the preservation of glomerular filtration by avoiding the hydrostatic capillary pressure decay, while decreasing the RPF. This ATII dependency is more prominent when the renal perfusion pressure is substantially reduced. Therefore, the increased levels of ATII that occur with blood volume depletion help preserve glomerular filtration and the excretion of metabolic products, such as urea and creatinine, which depend on glomerular filtration.

   Experiment 3. Afferent and efferent arteriole vasoconstriction and sympathetic nervous system action. Renal blood flow is determined by the arterial systemic pressure. The reduction in flow is detected by cardiopulmonary baroreceptors, which activate the sympathetic nervous system with direct neural activation of the arteriolar smooth muscle (noradrenaline action), as well as adrenal secretion by the adrenal gland. Most of the renal vascular resistance is on the EA and AA. The sympathetic neurohumoral activation promotes vasoconstriction of both arterioles; however, norepinephrine and epinephrine cause vasoconstriction by binding to the α1-adrenergic receptors, located mainly on the AAs. The activation of these receptors causes a decrease in the GFR and the renal blood flow (7).

   This experiment aims to simulate the action of the sympathetic nervous system on the renal arterioles. Follow the instructions below:
   1. Keep the water column on the EP.
   2. Adjust the claw gripper over the AA and EA tubes.
   3. Proceed with six complete rotations of the claw gripper on AA and one rotation on EA.
   4. With the beakers, measure the fluid volume flowing through EA and BC, simultaneously and without fluid loss, for 10 s.
   5. Record the values of the volume/flows in 10 s below:
      Renal plasma flow (the sum of the glomerular filtration rate and the flow through the efferent arteriole): ___________________
      Glomerular filtration rate: _____________________________
      Filtration fraction: _____________________________

DISCUSSION

The model uses relatively low-cost, everyday materials. We used the model to show the principle of mass conservation in the solution of an elementary bifurcation problem, as shown in Fig. 2, to obtain the 20% filtration fraction.

As mentioned above, it is important to keep the plumbing completely filled with water before using the system, because the calculations are dependent on there being no air bubbles in the flow and the flow being predominantly linear and without capillarity. This means that the tube dimensions should be large compared with the fluid density. According to the continuity Eq. 1, we have:

\[ A_1 v_1 = A_2 v_2 + A_3 v_3 \]  

where \( A \) is the cross-sectional area, and \( v \) is the flow velocity. It was stipulated that the BC flow rate is a fraction, \( p \)

Fig. 2. Elementary bifurcation scheme. \( Q \) represents the flow through each extremity, determined by the cross-sectional area \( A \) and the fluid flow velocity \( v \).

Glomerular filtration rate: _____________________________
Filtration fraction: _____________________________

Glomerular filtration rate: _____________________________
Filtration fraction: _____________________________

Glomerular filtration rate: _____________________________
Filtration fraction: _____________________________
(0 < p < 1), of the AA mass flow. In this case, the rest will be drained by EA. Therefore, the Eqs. 2 and 3 are:

\[ A_2v_2 = (1 - p)A_1v_1 \]  
\[ A_3v_3 = pA_1v_1 \]  

However, the tube dividing is the same as the tube expanding and, therefore, disregards any edge effect or compressibility. It is expected that \( v_2 = v_3 \), and, once the mass entering AA ends, it should split by the two drain tubes. Thus, dividing Eqs. 2 and 3, we have Eq. 4:

\[ A_2/A_3 = (1 - p)A_1V_1/pA_1V_1 \]  
\[ A_2/A_3 = (1 - p)/p \]  

The \( p \) filtration fraction is 20%, and the valid ratio \( A_2 = 4A_3 \) (Fig. 3) is represented below:

\[ A_2/A_3 = (1 - 0.2)/0.2 \]
\[ A_2/A_3 = 0.8/0.2 \]
\[ A_2/A_3 = 4 \]
\[ A_2 = 4A_3 \]

In our model, the ratio \( A_2/A_3 \) to \( p = 0.2 \) was obtained with the internal diameters 0.6 cm and 0.3 cm in the EA and BC output extremities, respectively. For practical purposes, we used tubes successively coupled to reach the 0.6-cm and 0.3-cm final internal diameters, although it is possible to use a single tube. Silicone tubes, or any other material resilient enough to minimize the deformations caused by the repeated compressions, can be used. The calculations do not consider the loss of energy along the course, so the Y bifurcation angle does not interfere with the experiment, nor do the tube lengths. In short, if the largest output extremity is twice the diameter of the smaller extremity, the flow rate through the latter will always be 20%.

The experiment starts with a demonstration of the parameters used to measure glomerular filtration. When this stage is finished, the students are introduced to the concepts of GFR, renal blood flow, and filtration fraction, as well as the GFR determinants based on Starling forces that act on the glomerular conduit. Of these forces, the most variable and susceptible to physiological control are the capillary hydrostatic pressure and the colloidal osmotic pressure (3). The capillary hydrostatic pressure variations are the primary determinant in the regulation of glomerular filtration. However, the model was designed as a hydraulic system and, therefore, does not demonstrate the colloidal osmotic pressure variations of either the glomerular capillaries or the Bowman space. Therefore, between the biophysical forces, hydrostatic is the only one represented by the apparatus.

Thus the model cannot demonstrate the hydrostatic pressure along the glomerular capillary achieving filtration equilibrium (2), nor the tubular reabsorption that is also determined by vascular resistance variation in the arterioles. Due to its simplicity, the model cannot illustrate concepts such as the filtration barrier and the filtration coefficient. However, it does allow the demonstration of pathological situations, such as postrenal injury caused by nephrolithiasis, ureteropelvic junction obstruction, or extrinsic tumor compression of the urinary tract by glomerular extremity (EA) obstruction or constriction. The observation of these phenomena is interesting to students in the healthcare area. The filtration fraction is another basic concept demonstrated in experiment 1. It was possible to see that only one-fifth of the blood flowing through the kidney is filtered by the nephrons.

Experiment 2 demonstrates an important action of AII on the renal circulation: EA vasoconstriction. At this point, we can expatiate about the lower sensitivity of the AA to AII, the counterfactual roles of prostaglandins and nitric oxide, and the specificity of ATII receptor subtypes; we can even simulate the pharmacological impacts of nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, or angiotensin receptor blockers. The experiment shows the intrarenal action of ATII, but it is possible to expand the discussion with the students to include its vasoconstricting role in the systemic circulation, in body fluid maintenance, and arterial pressure, which are also covered in experiment 3. This experiment also allows the visualization of the water column ascending in the carboy, which illustrates the principle of body fluid conservation. Understanding this principle is important for the full comprehension of the renin-angiotensin system.

In a normal, healthy individual at rest, the sympathetic tone seems to have little influence on the renal blood flow (3), meaning the baroreflex sympathetic activation resulting from body postural changes, for example, does not significantly affect the vasomotion of renal arterioles (8). From experiment 3, the intense action of catecholamines during, for example, strong emotional stimuli (e.g., fear, pain), dehydration, cerebral ischemia, or severe bleeding, should be clear to the students. Therefore, the last experiment evoked a hypovolemia situation to exemplify the synergistic action of the sympathetic system and renin-angiotensin. Hypovolemia was chosen because it represents the extravascular compartment contraction, which is also a physiological consequence of several clinical situations (e.g., diarrhea, vomiting, bleeding).

It is also known that the AA and EA smooth muscle reacts to a myriad of endocrine, paracrine, and neural stimuli, but the model only represents the most important (15). The model cannot simulate, for example, arteriole dilatation, even though prostaglandins, nitric oxide, and natriuretic peptides act to produce this effect. Although we tried to create an easy model,
it can be expanded into a more sophisticated hydraulic system and include more physiological concepts.

In the University of Caxias do Sul, more traditional biomedical teaching methods predominate, based on education by subject compartmentalization, rather than integrative methods. Although the model described in this paper has been introduced, the individual class pattern persists. It is evident that students have difficulty making connections between the subjects and transferring basic science to clinical learning or even to professional practice (16). With the development of this model, we aimed to minimize these obstacles and to provide an interactive demonstration of the physiological concepts, combined with clinical understanding, and to provide morpho-functional, pharmacological, and pathophysiological connections by active learning.

The experimental protocol contains all of the instructions necessary to handle the apparatus, as well as the physiological concepts behind each experiment, to ensure the model is easily manageable by students. Providing lessons that allow student participation is a more effective way of teaching, compared with passive learning (11). Supporters of this type of method believe active learning is more fun, motivating, and leads to the best information retention, and, therefore, exam performance (12). Studies also show that first-year medical students seem to prefer learning modules that involve multiple learning styles (10), and it is likely that they would have particular interest in teaching models that allow them to learn using real-life examples, case studies, or experiments that focus on student interaction (6). Despite being computer animations that offer a good understanding of renal concepts, they are still relatively passive ways of learning. The model introduced in this article provides active learning, resulting in a better understanding of the concepts. Through the model, the students can build a kidney and engineer and regulate its functioning; they do not just see the glomerular process but become active participants in the process. It is essential to emphasize, however, that these active methods work best when students regulate and monitor their own learning, an important aspect of this approach.

Conclusion

Based on a simple concept, the model presented here demonstrates the fluid redistribution that occurs within the glomerulus, due to arteriolar vasomotion. It was shown, based on this process, how selective changes in AA or EA resistance affect the GFR, a topic in glomerular hemodynamics that often confuses students beginning their renal physiology studies.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.C., R.A.S., L.d.S.R., and G.J.L.G. conceived and designed research; R.C., R.A.S., L.d.S.R., and G.S.F. performed experiments; R.C., R.A.S., L.d.S.R., and G.J.L.G. interpreted results of experiments; R.A.S. prepared figures; R.C., B.T.T., and G.S.F. drafted manuscript; R.C., L.d.S.R., B.T.T., G.S.F., and G.J.L.G. edited and revised manuscript; R.A.S., L.d.S.R., B.T.T., G.S.F., and G.J.L.G. approved final version of manuscript.

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