

## **ACUTE ANIMAL EXPERIMENT PROTOCOL (RENAL FUNCTION)**

1. Prepare 1% Albumin saline. (Usually prepare 100 or 200 ml in saline solution using a sterilized bottle. After filtered, which can be used for a week). Filtration to remove particles is very important!! Bovine albumin is pretty expensive, and use of 1% albumin solution is OK if surgical preparation does not have too many bleedings. (This is often done during a period waiting for equilibration, not everyday morning. It may take about a half hour to stir for mixing and then filter).
2. Prepare infusion syringes  
30 ml syringe for i.v. infusion (20-25 ml 1% albumin solution, then 1.5 to 3 ml/hour).  
5 ml syringe for renal arterial infusion if 1.5 ml/hour saline is infused. For 3 ml infusion, 30 ml syringe will be used.  
3 ml syringe for renal medullary infusion. (0.5-0.6 ml/hour saline will be used)
3. Prepare catheters  
For femoral arteries (PE 50 polyethylen tubing with #24 needle connector, or PE 60 tubing #21 needle connector. All catheters will be prepared by tapering the tip under flame)  
For femoral vein (same as arterial catheters)  
Renal Arteries (PE 10 tubing with #27 needle connector, tapered tip; but not too thin, otherwise it will be easy to be blocked due to stopping infusion pump during the experiments time.  
For pressure-natriuresis: need an extra arterial catheter into the carotid artery to record high level blood pressure (160 and 120 mmHg).  
For urine collection, prepare two catheters (using PE 50 or PE 60 tubing with a tapered tip)
4. Prepare heparin saline solution for flushing catheter to block blood clotting in the arterial catheters. Usually add 0.2 ml heparin stock solution into 20 ml saline in a 50 ml beaker.
5. Anesthetize rats.  
First, 0.02ml/100g body weight Ketaject injection into muscle; 3-5 min later, I. P. inaction (0.1 ml/100g, (stock solution: 1g in 20ml). (To prevent injection into intestine, use 1 ml syringe with #27 needle which is wrapped by a 5-7 mm long PE 10 tubing to shorten the length of this needle. About 5 mm long needle tip out of the PE tubing is used for injection).
6. Shave rat hair, clean and sterilize an area for surgical operation using iodine and then alcohol.
7. Rat is placed in a warm board for surgical preparations:

- a. Isolate femoral vein and insert vein catheter;
- b. Isolate femoral artery and insert arterial catheter;
- c. Isolate carotid artery and insert an arterial catheter if pressure natriuresis will be done;
- d. Open rat abdomen along the midline;
- e. Isolate renal artery from vein for flowmeter probe; (For renal function study, it is necessary to use some phenol to denervate renal nerve. Usually put 1-2 drops phenol around the aorta of both renal arteries (around 15 mm area) and then remove it in 30-60 seconds by wiping out with cotton tips). Renal infusion pump should be on all the time to keep catheter in patency.
- f. Renal arterial catheter. From another femoral artery, put a catheter into renal artery under watching catheter tip stained with dark black marker pen.
- g. Then place flowmeter around the renal artery, check zero point by clamping renal artery upstream of probe.
- h. During the surgery, monitor arterial pressure; when flowmeter is set, record renal blood flow.
- i. Insert catheters into each ureter, wait for about 1 min to see urine coming out.
- j. Add some saline drops on kidney surface and use parafilm to cover the surgical incision including all the area. (Remember, if use mineral oil, parafilm will be dissolved and therefore cannot be used).
- k. Equilibrate for 1-1.5 hour by infusion of i.v. 1% albumin saline at 3 ml saline/hour/rat.
- l. During the surgery and equilibration time, closely watch arterial pressure. If higher than 130 mmHg, more Inactin is needed to add by venous i.v. line for a stable anesthesia. Usually take PE tubing out from needle connector and add 2-3 drops inaction (about 10 cm length filled with inaction) and then connect back and push by infusion pump. Blood pressure will drop and rapidly recover in 2-3 min, but stay at a lower level. But blood pressure cannot be lower than 105 mmHg.

Now it is time to have lunch!!!

- m. After lunch, the rat should be still in the equilibration period. Take this time to add your notes in notebook (should have some notes before surgery about this day experiments, anesthesia and so on); Also need to label and weigh tubes for urine collection, write down all tare weight, real weight and net weight in the note book.
- n. Experiments: Any renal or i.v. acute drug protocol needs:
  - Control period for collection of urine, monitoring AP and RBF for 20-40 min. Remember, 5-10 min are need to remove dead space in the tubing and drug mixing and stabilizing its effect. For renal medullary infusion, 15-20 min are needed.

Real experimental period, 30 min for each dose (at least 5 min dead space and 20 min collection);

To determine in vivo doses, usually it is needed to try one effective dose first (with obvious effect but no leakage into circulation by seeing no change in blood pressure or urine flow in contralateral kidney) and then double and half reduce infusion speed to get the other two doses (lower and higher one than effective dose); After experiments, calculate nmol or pmol/min/100 g infused.

Parameters:

**RBF:** 6-10 ml/min

**BP (AP):** 105-120 mmHg, if BP is lower than 95 mmHg, there is no way to do renal function studies.

**U.V:** 10-15 ul/min/g kidney weight

Analyze Na<sup>+</sup> and K<sup>+</sup> by flame meter, calculate umol/min/g kidney weight.

Renal arteries perfusion drug concentrations:

3 ml/hour=3000  $\mu$ l/60 min = 50  $\mu$ l/min

50 $\mu$ l /10000  $\mu$ l (RBF 10 ml) = 1:200 dilution

If stock solution is 200 mM, renal blood concentration is 1 mM;

If stock solution is 20 mM, renal blood concentration is 100  $\mu$ M

If stock solution is 2 mM, renal blood concentration is 10  $\mu$ M

o. Confirmation experiments:

For dilation or increase in RBF: infuse known dilators such as sodium nitroprusside (see N Parahk/Zou's paper for dose)

For contraction, infuse Ang II 10 ng/min/100g body weight.

If these known compounds have no effect on RBF, it is indicated that the animal somehow is not responsive or preparation is not working.