THE UPPER URINARY TRACT

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There is no evidence of any change in the composition of urine as it traverses calyces, pelvis and ureter. The sole function of this tract is to convey urine from kidneys to bladder yet it plays a far more active role than that of a simple conduit. Flow down the tract is encouraged by repeated series of co-ordinated muscular contractions loosely referred to as peristalsis.

The major issues in the physiology of the upper urinary tract have been (a) the role of the intrinsic nerve plexus in propagation of the peristalsis-like ureteral contractions, (b) the nature of the stimulus responsible for the initiation of these repeatedly occurring contractions and (c) the influence of extrinsic nerves upon this ureteral activity.

In order to throw some light on these problems, physiologists and pharmacologists have for many decades tried to develop objective methods by which the activity of the upper urinary tract can be monitored. The methods available involve either *in vivo* or *in vitro* studies of both human and animal responses.

Effect of drugs

Smooth muscle of human upper urinary tract contains cholinergic and adrenergic receptors. The latter can be subdivided into excitatory α - and inhibitory β -receptors. Both noradrenaline and acetylcholine elicit an increase in resting tension and contraction frequency whereas isoprenaline and phentolamine each inhibit spontaneous contractions. Atropine has no effect on spontaneous contractions at doses which abolish contractions induced by acetylcholine. Most animals species investigated, including cat (Lucas, 1908), dog (Roth, 1917; Malin *et al.*, 1968), pig (Longrigg, 1974a & b; Muschat, 1929), rabbit (Gosling & Waas, 1971), rat (Finberg & Peart, 1970) and water-buffalo (Gould *et al.*, 1955), have been shown to possess excitatory α -adrenoceptors as in human tissue. Less consistent responses have been found when using β -adrenoceptor- and muscarinic-agonists. In the pig there is conclusive evidence for inhibitory β -adrenoceptors and excitatory muscarinic receptors throughout the whole of the upper urinary tract. In man, β -adrenoceptors have not been demonstrated above the pelvi-ureteric junction. Although the adrenoceptors do receive a functional innervation, there is no such innervation of the muscarinic receptors (Longrigg, 1974b). Other species such as dog

and water-buffalo have been shown to respond to muscarinic agonists although 'to a lesser degree than seen with α -adrenoceptor stimulation' (Gould *et al.*, 1955; Malin *et al.*, 1968).

Human studies *in vivo*

Experimental method

An array of conflicting results has been obtained using different methods of investigating the intact ureter in various species. The technique to be described aims at producing an objective method of evaluating motor responses of the human intact ureter to various pharmacological agents.

Basal intrapelvic and intraureteral pressures and their phasic pressure changes are measured by means of indwelling non-obstructing fluid filled catheters. These recordings can of course only be made during an investigation which requires the passage of ureteral catheters for diagnostic or therapeutic procedures.

The patients have oral fluid withheld for approximately 4 h and are given detailed explanation of the procedure, the information it may provide and how this may help in their future management. While still in the ward a small strain gauge force transducer is strapped around the chest and the patient is then transferred to the operating theatre. Here, under aseptic conditions, and with the patient in the semi-lithotomy position, the bladder is first examined endoscopically. A number 3 or 4 Ch ureteric catheter the lumen of which has been filled with isotonic saline at body temperature is then introduced and advanced until its tip is thought to be lying in the renal pelvis. By passing a fluid filled catheter one hopes to avoid drainage of urine from the renal pelvis during insertion and the subsequent disruption of existing pelvic pressure relationships. The cystoscope is next carefully removed and the position of the catheter tip checked by a plain radiograph. Appropriate monitoring equipment allows graphic recording of the pressure complexes occurring within one or both ureters, together with parallel changes in respiration (Figure 1). The catheter can be withdrawn so that its tip lies at any chosen point along the length of the ureter. The position of the catheter hole (end or side) has little influence on the results (Longrigg, 1974a). One major criticism is that the introduction of a foreign body into the ureter may itself cause

Figure 1 Method of monitoring pressure changes occurring in the human ureter.

abnormal contractions and for this reason a 3 Ch catheter is preferred. Using such a small bore catheter produces damping of the system but it is considered adequate for the type of pressure complexes recorded from the human ureter. Great care must be taken to remove all air bubbles from the system prior to recording in order to ensure that damping is not excessive.

Types of activity observed in the human ureter

Under the conditions described the total urinary output is approximately 50 to 120 ml/h. On making the connection between ureteral catheter and pressure transducer, immediate recordings are obtained of the phasic intraureteral pressure changes. These are initially irregular in rate and amplitude but after

approximately 12 min a regular pattern develops. With the tip of the catheter in the upper third of the ureter, the average rate of contraction complexes varies from one to 10 per min. Taking the symphysis pubis as a reference point the basal pressure ranges from 3 to 15 cm of water and the change in pressure in response to a contraction wave varies from 10 to 70 cm. Bilateral recordings show that each side functions independently; the pressure changes are different and not in phase.

The dramatic inhibitory effect of isoprenaline on this activity is illustrated in Figure 2. Atropine or propantheline at doses which produce unequivocal evidence of blockade of muscarinic cholinceptors in other organs have no effect on the spontaneous contractile activity of the human upper urinary tract.

Limitation of human studies

Although this measurement of urinary tract activity is simple and relatively safe, it is obviously invasive and can cause morbidity. It therefore cannot be used as a routine method for assessing the effect of drugs. The other criticism is that the presence of even a small bore catheter may modify ureteric activity. This interference is avoided by the technique of passing a percutaneous cannula through the renal parenchyma directly into the pelvis (Whitaker, 1979).

Ureteric activity is influenced by the rate of urine flow (volume per unit time) so that diuretic drugs have indirect effects. An increase in flow provokes only a small increase in the frequency of transmitted contractions which usually remains within the range 1–5 min. The increase in flow is accommodated rather by increase in the volume of the transmitted bolus of urine (Constantinou & Djurhuus, 1982). Deliberate increase in the rate of urine flow can be achieved with frusemide or i.v. mannitol. It is difficult however to standardise the rate of urine flow or indeed to *measure* the flow down one ureter in man. For this reason some investigators directly infuse saline at a known rate through a cannula in the renal pelvis.

The transmission of the urine bolus and its associated pressure waves from the pelvis to the bladder down the ureter is accompanied by a propagated wave of electrical activity. The pelvic or ureteral electromyograph can be recorded from platinum electrodes sutured to the walls of the tract at open surgery. Hanley (1953) developed a method of measuring in man the electrical activity which accompanies ureteral contractions. Such measurements were made by means of non-occlusive catheters attached to the end of which were small silver collar electrodes. A similar technique was employed by Draper & Zorngiotti (1954) in studies on the intact canine ureter. Artifacts are readily produced and the technique has not gained much popularity compared with the measurement of pressure.

Figure 2 Spontaneous pressure changes recorded from a catheter lying with its tip in the upper third of the human ureter. Isoprenaline infusion (i.v. $10 \mu\text{g}/\text{min}$; ▲ to ○) produced inhibition of contractions and a fall in basal pressure.

Clearly the invasive techniques are applicable only to patients requiring detailed investigation for suspected disorders of pelvi-ureteric motility. Any measurements of drug response will be opportunistic. Systematic evaluation of dose/response relationships or cross over comparisons between different drugs are scarcely practicable.

A non-invasive technique potentially applicable to drug studies uses the gamma camera to map the excretion renogram. The patient receives i.v. ^{123}I -hippuran or $^{99\text{m}}\text{Tc}$ -diethylenetriaminepentaacetic acid (DTPA). The radionuclide is detected as it passes from blood to renal parenchyma then down the upper urinary tract to the bladder. The radiation dose is roughly comparable to that for a plain chest X-ray. The isotopes have short half lives and the labelled compounds themselves are rapidly eliminated. It might therefore be practicable to investigate the influence of a drug on kidney to bladder transit times for example. Tests on patients could be repeated under different conditions at intervals of a day or two. When necessary the resolution of the renogram could

be improved by deconvolution analysis (O'Reilly & Lupton, 1982).

Most of the information about *in vivo* drug effects has hitherto been obtained from animal studies.

Animal studies *in vivo*

Reasons for selection of pig

The dangers of extrapolating the results of animal experiments to man are well known. One would like therefore to use the experimental animal with the urinary tract anatomy and physiology most like man. Other factors need consideration in particular availability and cost. As early as 1919 (a, b) Satani remarked on the similarity of the ureteral musculature in both pig and man and this has since been confirmed by several authors. Melick *et al.* (1961) compared the urinary tracts of pig and man by cystograms, cystometrograms and intra-ureteral pressure recordings and were unable to detect any significant differences.

Pressure and flow measurements

Upper urinary tract motility in the pig can be measured in a similar way to that described for man. Eight-week old female pigs are used. They are deprived of food and water for approximately 3 h prior to anaesthesia by i.v. sodium pentobarbitone (25 mg/kg). This dose does not have any recognised effect upon upper urinary tract activity. Tracheostomy is immediately performed and a number 6 cuffed endotracheal tube inserted. This is to avoid airway obstruction and not to deliver an inhalational anaesthetic. Anaesthesia is maintained by further intravenous doses of pentobarbitone. The animal is placed supine on the operating table, cleansed and draped to expose the abdomen and the left groin. A midline incision is made from xiphisternum to pubis and with suitable retraction each upper ureter is exposed. The activity of the ureters is measured by inserting fluid filled catheters and recording the phasic pressure changes. The ureteric orifices are very small however and awkwardly placed low down in the bladder. Even number 2 Ch catheters partially occlude the lumen causing dilatation of the whole ureter and obvious disturbance of 'normal' intra-ureteral pressure. The following method is therefore adopted. The ureter is identified at a point 3 to 4 cm above the bladder, its overlying peritoneum is incised and with minimal mobilisation the ureter is completely transected in an oblique line. A number 2 Ch Portex nylon cannula filled with isotonic saline at approximately 37°C is now introduced into the proximal orifice and advanced up the ureter so that its tip rests in the desired position. The end of this catheter is connected to a venous pressure transducer which in turn gives permanent recordings of intra-ureteral pressure changes on a potentiometric recorder.

An exactly similar procedure on the contralateral ureter allows bilateral simultaneous recordings to be made. Through an incision in the exposed groin, the common femoral artery can be cannulated to allow simultaneous measurement of blood pressure.

For estimating ureteral urine flow rates, a 1 cm length of a large bore (number 6 Ch) catheter is secured in the distal few mm of the proximal ureter and allowed to drain into a graduated receiver over a noted period of time.

Ureteric nerve stimulation

This is achieved by means of a generator which produces square wave pulses of varying current, frequency and duration. To make contact with the individual ureteral nerves, a specially constructed electrode is employed. This consists of two rectangular areas of platinum foil ($4 \times 1 \times 0.025$ mm) kept apart at a distance of 2 mm by means of Bostick quick set epoxy adhesive. One surface of each elec-

trode is coated with a thin film of the same adhesive. The tip of each electrode can be bent back upon itself and because of the thinness of the platinum it can be squeezed into position around the nerves to maintain close contact.

Types of activity observed

Under the described conditions the rate of urine flow down each ureter is between 0.1 and 0.2 ml/min and the propagated contraction waves are seen to coincide with the recorded pressure complexes. These complexes occur at a frequency of 1 to 3 min with amplitudes ranging from 4 to 12 cm of water. The basal pressures remain remarkably constant except when the catheters are disturbed by the violent expirations often noted in animals anaesthetised for more than 1 h. Using this technique it is possible to observe the effects on ureteric activity of both nerve stimulation and systemic drug administration.

Limitations of in vivo studies

Despite the relatively reproducible recordings obtained both in man and pig, so many uncontrolled variables operate *in vivo* that quantitative examination of drug response is fraught with difficulty. More completely controlled conditions can be provided only by *in vitro* studies of isolated urinary tract muscle.

Isolated tissues in vitro

Availability of human tissue

Human tissue can be obtained from patients being treated for neoplastic conditions of the upper urinary tract. The tissue is immediately placed in Krebs's solution at approximately 37°C in a portable carrying outfit which allows the fluid to be gassed with 5% CO₂ in oxygen.

Many of the smaller animals previously studied have a uni-calyceal collecting system. This means that the kidney has one single papilla projecting into one large calyx. The kidneys of the pig provide a situation more analogous to that of man in that the pig kidney has a multi-calyceal system with 4 or 5 papillae projecting into a corresponding number of minor calyces.

Preparation of specimens

The ureters are cut across immediately below the pelvi-ureteric junction. The following method is used for taking specimens of calyx. After removing the ureter one blade of the dissecting scissors is introduced through the orifice of the ureter into the renal pelvis and a cut is made through the medial border of the kidney substance first to the upper pole and then

to the lower. The kidney can then be opened like the leaves of a book to reveal the renal papillae projecting into each calyx. The distal half of a selected papilla is then removed with a scalpel in order to expose fully the forniceal region. A strip of calyceal tissue approximately 1 cm by 3 mm is then carefully dissected from around the base of the papilla in a circumferential manner. In order to be sure of taking the most proximal calyceal tissue, a narrow border of papilla base is included in the strip. If this last step is omitted, the strip will tear as its most proximal attachment and leave behind the important forniceal area of the calyx. From the same kidney it is possible to obtain circularly orientated strips of pelvic muscle.

Setting up the preparation

Using 6/0 atraumatic Mersilk sutures the tissue is suspended in a 20 ml jacketed organ bath containing Krebs's solution at $38 \pm 0.5^\circ\text{C}$ and gassed through a glass scinter with 5% CO_2 in oxygen. Changes in tension are measured isometrically in the usual manner. Approximately 300 mg resting tension is applied to the specimen and a period of 15 min

allowed for stabilisation before the experiments are begun. Using pig tissue, 6 min cycles can be used (2 min control, 2 min drug contact and 2 min wash). On human calyx specimens and pig renal pelvis, this cycle has to be extended when using certain drugs.

Two cm segments of ureter can be suspended longitudinally in a manner similar to that described for the calyces. Resting tension is adjusted to approximately 500 mg.

The above method results in a preparation which gives regular spontaneous contractions against which the effects of drugs can be measured. However, as one passes down the upper urinary tract, spontaneous activity becomes less regular and specimens from the lower third of the ureter remain totally inactive unless stimulated. The following method may be used for obtaining rhythmical contractions from this area of the urinary tract. It is based on the classical Trendelenburg technique used originally for the study of intestinal activity in small animals and is represented diagrammatically in Figure 3. The usual tissue pillar is replaced by a hollow glass tube over the curved end of which can be pulled the distal 3 mm of the specimen. This end of the tube is slightly flared at its tip so that

Figure 3 Isolated segments of ureter from the lower third do not contract spontaneously. Regular rhythmical contractions may however be provoked by mounting the segment in this modified Trendelenburg apparatus and raising the reservoir to give a pressure head of 5–10 cm water.

the ureter can be secured by a single 5/0 silk suture. Proximally the ureter is closed by a ligature of similar material which is then attached to the force transducer. The glass tube and attached ureter both containing Krebs's solution are then connected via a three-way tap to a reservoir of the same solution, the height of which can be adjusted to as to vary the intra-ureteral pressure. By raising and lowering the reservoir, fluid can be made to flow into or out of the closed segment of ureter. If the pressure in the latter is adjusted to approximately 5 to 10 cm water, then in response to distension, the ureter will contract to expel the fluid back into the reservoir system and on relaxation will again accommodate a similar quantity of fluid. Segments of normally inactive lower ureter are in this way coaxed into rhythmical activity as fluid see-saws backwards and forwards from ureter to reservoir. Each contraction is accompanied by change in longitudinal tension which is monitored by the force transducer. A fine polythene tube taken from the three-way tap to a venous pressure transducer allows simultaneous recordings of the intraluminal pressure changes.

Both the above methods can be adapted in order that transmural electrical stimulation may be applied to the specimen.

Types of activity observed in isolated tissue experiments

All preparations of *human minor calyx* exhibit immediate spontaneous tension changes occurring at rates of 10 to 13 per min with amplitudes varying from 200 to 600 mg. The frequency, amplitude and rhythm of the contractions are extremely regular and left undisturbed such tissues will remain spontaneously

active for well over 4 h. If the resting tension is increased to 500 mg, the tissues respond with contractions of greater amplitude but of varying frequency. These changes are transient and by a process of active relaxation the status quo is resumed within a few minutes. Within moderate limits there appears to be no relationship between resting tension and the final form and rate of contraction of smooth muscle from this area of the upper urinary tract. Figure 4 demonstrates how such spontaneous contractions may be influenced by the addition of adrenaline, noradrenaline and phenylephrine. Drug induced increases in resting tension show conventional concentration/effect relationships (Figure 5), (Longrigg, 1975).

Preparations of pig renal calyx exhibit similar regular rhythmical changes in tension in the range of 200 to 1,000 mg and at rates of 5 to 9 min for over 6 h. After initial interference with the fresh tissue either by drug addition or by electrical stimulation, some preparations exhibit a greater rate of spontaneous activity. This remains stable for the duration of the experiment and allows quantitative experimentation with both excitatory and inhibitory drugs.

Human renal *pelvis* and *major calyx* preparations show a striking similarity in their behaviour. Only a minority of preparations exhibit spontaneous activity (rate of approximately 4/min). Most remain completely quiescent for up to 1 h and cannot be coaxed into rhythmical activity even by the use of relatively large doses of adrenaline or noradrenaline. They respond to stimulatory drugs by an increase in tension with or without occasional superimposed contractions. They are however, unsatisfactory for quantitative pharmacological work in that repeated washing of the tissues after drug addition does not result in a reproducible return to basal tension.

Figure 4 Spontaneous tension changes recorded from an isolated strip of human minor calyx. The contraction frequency and basal tension are both increased by the addition of sympathomimetic amines to the bath fluid (1 $\mu\text{g/ml}$). Added at $\blacktriangle$; washed out at $\bigcirc$.
a adrenaline, b noradrenaline, c phenylephrine.

Figure 5 Log concentration-response curves for adrenaline (●), noradrenaline (○) and phenlephrine (■) acting on an isolated strip of human minor calyx.

Renal pelvis tissue from the pig resembles human minor calyx more than human pelvis. Spontaneous activity is more commonly observed and even when this is absent at first it can be initiated by brief exposure to adrenaline or noradrenaline.

Specimens taken from the upper third of the *human ureter* can be expected to give spontaneous contractions within 30 min of setting up the preparation, although it may be necessary to wait a further 15 min before such activity is regular enough to allow observation of drug effects. At this time, changes in tension of 1,000–3,000 mg are seen at intervals of 0.25 to 2 min. The interval tends to be constant in a particular specimen. Middle third ureter from man behaves in a similar way but lower third specimens are quiescent unless stimulated. Groups of contractions can be initiated by adrenaline at concentrations of 2 $\mu\text{g/ml}$ upwards but cease spontaneously before washing with Krebs'. Ureteric specimens from the pig behave in a very similar manner. The inactive lower third segments of ureter can be made to contract spontaneously by the closed segment technique (Figure 3). The act of expulsion of fluid is accompanied by tension changes in the length of the ureter and also by a most noticeable twisting movement of the tissue around its longitudinal axis. Tension changes reach up to 5,000 mg and occur at a frequency of about 6/min. These changes are modified by drugs. Adrenaline for example increases the fre-

quency but reduces the amplitude of the tension changes.

Conclusion

None of the described methods is ideal for evaluating the effect of drugs on upper urinary tract musculature. The catheter technique used to measure intra-ureteral pressures is probably the most reproducible *in vivo* method to date. Unfortunately it is directly invasive. It may occasionally be possible to gain informed consent for the trial of a particular drug in a patient who has an indwelling ureteric catheter but opportunities for experimentation of this nature are severely limited. Reproducible alternative methods of measuring ureteral activity are not yet available although the excretion renogram recorded by the gamma camera offers some promise.

The lack of non-invasive methods for investigating the intact human ureter is perhaps the main reason for the neglect of this area by clinical pharmacologists. For most human work the investigator must turn to *in vitro* methods. Problems are again experienced in acquiring suitable human tissue but if these are overcome by close collaboration between surgeon and pharmacologist such methods do provide an extremely reproducible way of investigating drug effects both quantitative and qualitative.

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