*Br. J. Pharmac.* (1970), **39**, 373–381.

# Function of smooth muscle of the rat renal pelvis—response of the isolated pelvis muscle to angiotensin and some other substances

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**Summary**

1. The circular component of the renal pelvis muscle of the rat appears as an intact band around the tip of the papilla.

2. The isolated pelvis muscle strip showed spontaneous rhythmic contractions in Locke solution at 38° C. The rate of contractions corresponded with the rate of pressure fluctuations recorded during free-flow from a ureteral catheter inserted up to the renal hilum of anaesthetized rats.

3. Angiotensin and adrenaline (13 ng/ml) produced a rise in tone of the isolated pelvis muscle, and an increase in the rate of spontaneous contractions; noradrenaline produced mainly an increase in the amplitude and rate of spontaneous contractions. Angiotensin and noradrenaline injected intravenously also produced an increased rate of pressure fluctuations recorded from the ureteral catheter.

4. Acetylcholine, nicotine and 5-hydroxytryptamine had no effect on the isolated tissue, but isoprenaline was capable of causing a decrease, and tyramine an increase, in the rate of spontaneous contractions in high dose (0·33 $\mu$g/ml).

5. An increase in the tone of the circular pelvis muscle could lead to a reversible obstruction of urine outflow from the kidney.

## Introduction

The presence of intrarenal smooth muscle was noted by Henle (1866), who ascribed the term "milking muscle" to the smooth muscle of the renal calyces on the basis of its situation and morphology. Movements of the calyces, or of the papilla itself, have long been observed (Engelman, 1869). In the young rat, the tip of the papilla extends just beyond the renal hilum, and can be observed directly *in situ* by dividing the upper ureter. Such an exposed papilla can frequently be observed to exhibit slow, rhythmic circular movements, which are caused by the contraction of surrounding musculature, since the papilla itself does not contain muscular tissue.

In a previous investigation (Finberg & Peart, 1970) we reported that angiotensin diuresis in the rat was occasionally accompanied by marked distal tubular dilatation at relatively low urine flow rates, indicating that collecting duct resistance to flow

could be increased by angiotensin. The fact that angiotensin possesses marked smooth muscle stimulant properties led to a consideration of the effect of contraction of the muscular tissue surrounding the tip of the papilla. Accordingly, an *in vitro* preparation of the muscle strip has been developed, similar to that described by Muschat (1929) in the pig, in order to study the effect on contraction of angiotensin and other agonists.

### *Morphology of the renal pelvis muscle*

The structure referred to as the renal pelvis muscle, or pelvic septum, is continuous with the upper ureter, and envelops the tip of the papilla, being loosely attached to the renal parenchyma by adipose tissue. Seen in longitudinal section, the pelvic septum has a crescentic border (Moffat & Fourman, 1963), the distance between the concave margins and the tip of the papilla being about 1·5 to 2 mm, in rats of about 200 g weight. The pelvic septum is supplied with blood by branches of the interlobar arteries and glomerular efferent arterioles (Moffat & Fourman, 1963).

When seen in transverse section, the pelvis muscle appears as a complete ring around the papilla (Fig. 1). In fixed preparations, shrinkage artefacts cause an

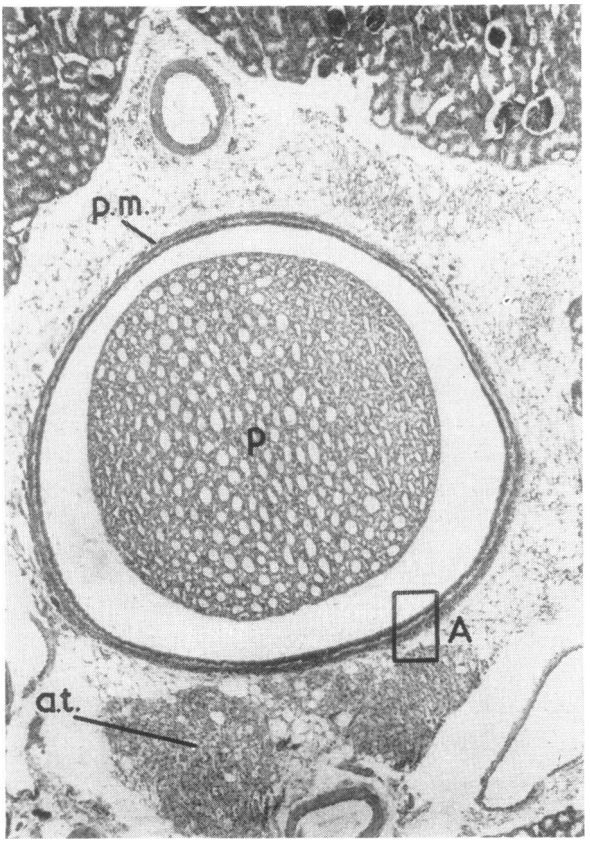


FIG. 1. Transverse section of rat kidney, about 0·6 mm from tip of papilla. p, Papilla; p.m., pelvis muscle; a.t., adipose tissue of sinus renalis. (Haematoxylin and eosin stain, ×*c.* 48.)

apparently large space between papilla and pelvic septum, although in fresh kidneys the two are closely applied. A short distance from the tip of the papilla, smooth muscle fibres, oriented in a circular plane, lie just under the epithelium and appear as an intact ring. Outside the circular band lie fibres oriented in longitudinal and oblique planes (Fig. 2). As the distance from the tip of the papilla increases, the discrete circular band becomes interrupted, and the proportion of longitudinal and oblique muscular components increase, as branches of the pelvic septum radiate out to cover the interlobar arteries and veins.

## Methods

Rats (200 to 300 g) were killed by a blow on the head and a kidney removed. The pelvic septum was exposed by a razor cut which hemisected the kidney in the sagittal plane, passing through the sinus renalis to one side of the papilla. The bulk of the parenchyma was separated by a transverse cut, and the papilla removed. A fine scissor point was passed into the pelvic cavity and so to the ureter, and the pelvic septum divided at one point, thus enabling the muscular portion to be opened out, and remaining renal parenchyma trimmed off.

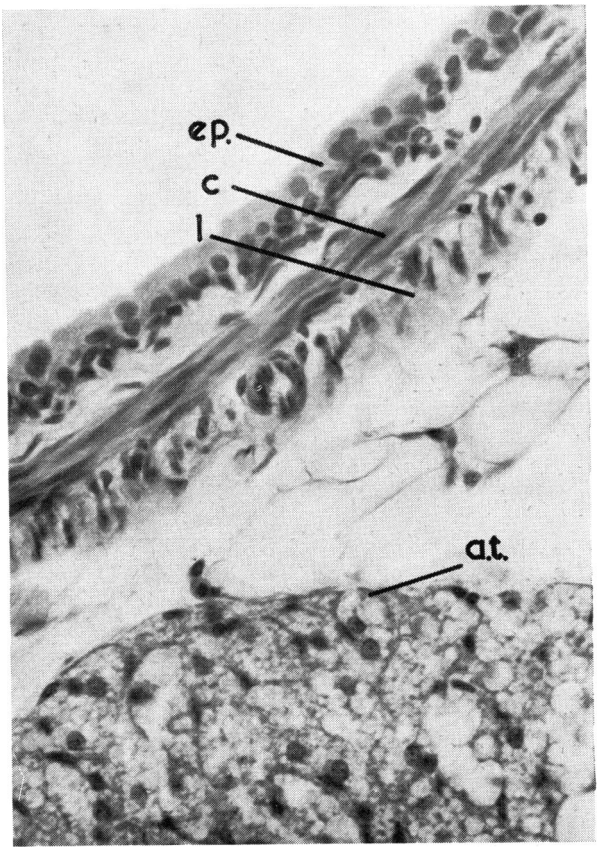


FIG. 2. High-power view of region A outlined in Fig. 1. ep, Epithelium ; c, circular smooth muscle ; l, longitudinal smooth muscle ; a.t., adipose tissue. (×*c.* 750.)

Using a fine atraumatic suture (Mersilk 7–0), the muscle strip was suspended in an organ bath (15 ml capacity) and attached to a calibrated Statham isometric strain gauge (±4·25 g maximum load) so as to record contractions of the circular component. Contractions were recorded on a Sanborn direct writing recorder. The preparation was placed in Locke solution, well gassed with 95% oxygen-5% carbon dioxide, or with pure oxygen, at 38° C, and given a resting tension of 50 mg. Following a 30 min equilibration period, drugs were added in a 3 min cycle allowing a 1 min contact time.

In four rats anaesthetized with 10 mg/100 g of Inactin (5-ethyl-5-(1-methylpropyl)-2-thiobarbituric acid) intraperitoneally, free-flow hydrostatic pressure was recorded from a ureteral catheter inserted up to the level of the renal hilum. The catheter was made from polythene tubing (internal diameter 0·29 mm, external diameter 0·61 mm) attached to a T-piece made from slightly larger tubing (internal diameter 0·5 mm, external diameter 1·0 mm) and the free end was kept in a saline reservoir, the surface of which was 1 to 2 cm below the renal pelvis. Pressure was measured in mmHg (1 mmHg≡1·33 mbar) from the side-arm of the T-piece with a Sanborn 267B pressure transducer coupled to a Sanborn recorder.

*Drugs*

Angiotensin in the form of valine[5] angiotensin II amide (Hypertensin, Ciba). (−)-Noradrenaline and (−)-adrenaline as acid tartrate; the amounts mentioned in the text refer to the base. Isoprenaline sulphate, tyramine sulphate, nicotine acid tartrate, 5-hydroxytryptamine creatinine sulphate, acetylcholine chloride; the amounts refer to the salts. Phentolamine as Rogitine (Ciba). The catecholamines contained 1 in 100,000 ascorbic acid.

*Composition of Locke solution*

NaCl, 9 g; $CaCl_2$, 0·2 g; KCl, 0·42 g; $NaHCO_3$, 0·3 g; glucose, 1 g; distilled water to 1 litre.

## Results

The isolated pelvis muscle commenced spontaneous contractions immediately on suspension in Locke solution. The amplitude of contractions was generally 20 to 50 mg at a rate of 10 to 18 per min (Fig. 3). Similar contractions, but of smaller amplitude, were obtained from the muscle strip suspended to record contractions of the longitudinal component.

Angiotensin, noradrenaline and adrenaline all caused an increase in the rate of spontaneous contractions. Angiotensin caused a marked increase in tone, and generally reduced the amplitude of spontaneous contractions. Adrenaline produced a similar response, but increased the amplitude of spontaneous contractions in most preparations. Noradrenaline caused an increase in the amplitude of spontaneous contractions, but only a slight increase in resting tension.

There was a great variation in sensitivity to angiotensin of different muscle strips, both in the minimum dose causing an effect as well as in the maximum tension developed. Most preparations showed a response to angiotensin at 13 ng/ml, but the increase in tone produced varied between 5 and 50 mg. Occasional preparations

showed only a poor response to angiotensin (67 ng/ml). The most sensitive preparation produced a distinct rise in tone to 1·3 ng/ml of angiotensin.

Preparations of the pelvis muscle in Locke solution gassed with oxygen/carbon dioxide mixture showed a diminution in the rate of spontaneous contractions after 1·5 to 2 h, and a fall in the pH of the medium to 6·5. Saturating the Locke solution with pure oxygen, however, produced a pH of 7·5 to 8·5, and no fall off in spontaneous activity even after 4 to 5 h. In such preparations, the increase in tone could be quantitated by measuring the average increase in relaxed tension, and dose response curves to angiotensin and adrenaline could be constructed (Fig. 4). Angiotensin was always more potent than adrenaline in increasing the tone of the preparation, but the ratio of potency between the two substances in six preparations varied between 1·7 and 13·5 (on a weight basis).

Both the increase in tone, as well as the increased rate of spontaneous activity, produced by adrenaline and noradrenaline were prevented by phentolamine (3·5 $\mu$g/ml) added to the bath 2 min before the catecholamines. The response to angiotensin was unaffected by this dose of phentolamine (Fig. 3).

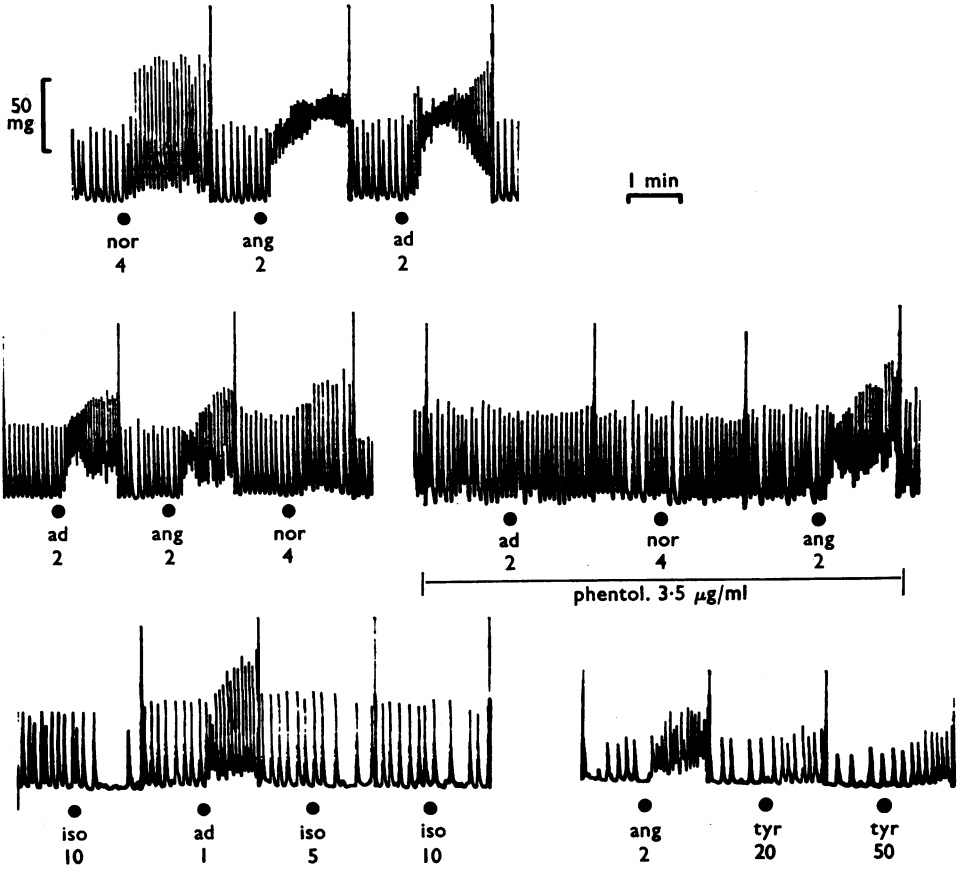


FIG. 3. Contractions of circular component of rat renal pelvis muscle in Locke solution at 38° C, gassed with 95% oxygen-5% carbon dioxide. Responses to angiotensin (ang), adrenaline (ad), noradrenaline (nor), isoprenaline (iso) and tyramine (tyr). Figures indicate amount of drug in $\mu$g added to bath (15 ml). Results from four experiments shown. The long vertical lines indicate where the bath fluid was changed. Phentolamine (phentol.) was added to the bath after each change of the fluid, in the right-hand part of the middle record.

In three preparations, isoprenaline was without effect on the pelvis muscle, but in two others a reduction in the rate of spontaneous contractions was produced by a large dose (0·33 and 0·66 $\mu$g/ml). Acetylcholine, nicotine and 5-hydroxytryptamine were without effect on the tissue, but tyramine produced an increase in the rate of spontaneous contractions at 0·33 to 1·3 $\mu$g/ml in three out of four preparations (Fig. 3).

The flow of urine from the kidney of anaesthetized rats showed fluctuations at a rate similar to the contractions of the isolated pelvis muscle, as indicated by the fluctuations in free-flow pressure recorded from the ureter (Fig. 5). Angiotensin and noradrenaline injected intravenously both caused an increase in the rate of pressure fluctuations, but angiotensin caused a fall followed by a rise, and noradrenaline produced a rise, in free-flow pressure. The changes in free-flow pressure were a reflection of the antidiuretic and diuretic effects of the two substances, but the increase in rate of pressure fluctuations indicated an increase in the rate of spontaneous activity of the pelvis muscle.

## Discussion

The role of intrarenal smooth muscle in assisting the expulsion of urine from the kidney must vary greatly between species, owing to the structural diversity of the renal pelvis and calyces. In addition, the inadequacy of the techniques available for investigating this problem has led to a variety of opinions on the interpretation of the results.

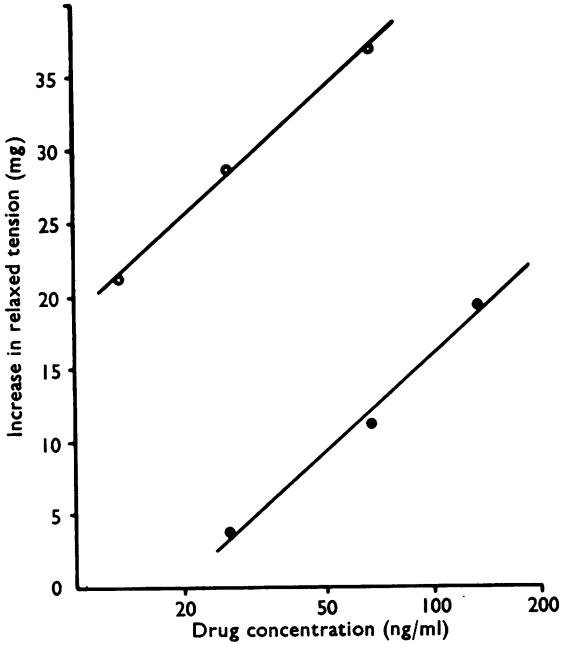


FIG. 4. Dose-response curves for the effect of angiotensin (○——○) and adrenaline (●——●) on increasing the tone of the isolated renal pelvis muscle of the rat (Locke solution at 38° C, gassed with pure oxygen). Ordinate represents maximum increase in relaxed tension during 1 min contact time, against drug concentration in ng/ml (abscissa). Each point the average of two or three drug additions on one muscle strip.

Muschat (1926), following one observation on haematuria in man, postulated that constriction of circular muscle around the papilla could cause venous congestion, and could possibly be responsible for reflex anuria. By making serial sections of the calyces, he found the smooth muscle to be orientated as a single shallow spiral, giving the appearance of two circular bands joined by an oblique strip. Muschat (1928) studied the pressure developed in single pig calyces suspended *in vitro* ; the calyx was found to expel its entire contents every 30 s. Subsequently (1929) he studied strips of spiral muscle from pig calyces *in vitro*, which showed spontaneous contractions at a rate of 10 to 12 per min, and found adrenaline to cause a marked rise in tone of the preparation. These observations by Muschat appear to be the only previous studies on the contractions of intrarenal smooth muscle *in vitro*.

Narath (1951), in a much larger investigation of human calyces, found that the circular muscle does not generally exist as an intact band around the papilla. Narath proposed that longitudinally orientated muscle bundles were able to protect the canaliculi from reflux, by elevating the fornix against the ducti Bellini during contractions of the pelvis. Kiil (1957), however, found that intrapelvic pressure never increased greatly during contraction of the pelvis, and argued against the necessity for such a mechanism.

In the rat, the close contact between pelvis muscle and papilla, as well as the similarity between the rate of contractions of the muscle strip *in vitro* and the rate of pressure fluctuations recorded from the ureteral catheter, indicates that the pelvis muscle may assist in expelling urine from the papilla during life. Steinhausen (1964) noticed rhythmic contractions of musculature at the base of the exposed hamster

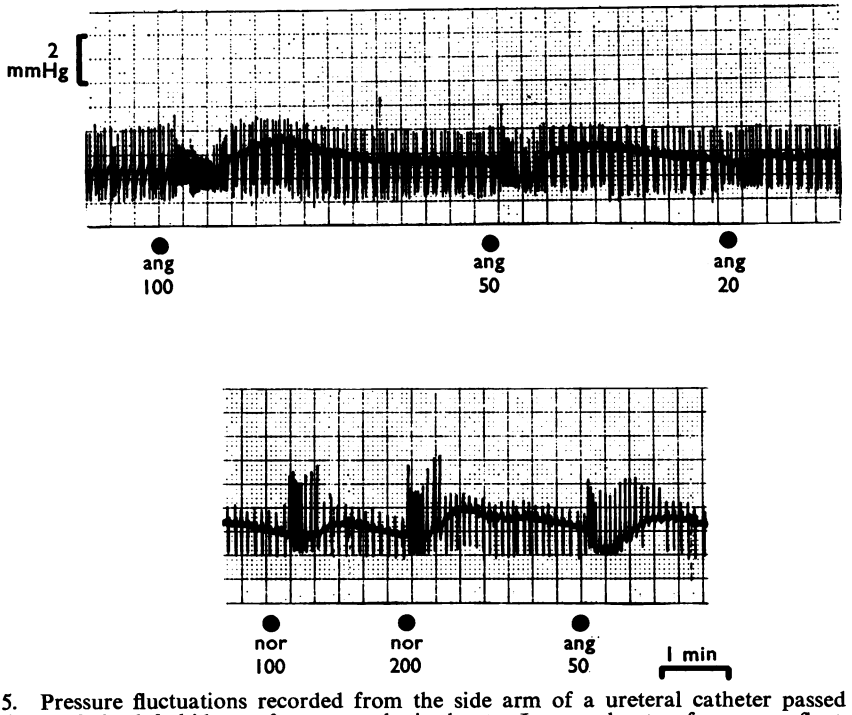


FIG. 5. Pressure fluctuations recorded from the side arm of a ureteral catheter passed up to the hilum of the left kidney of an anaesthetized rat. Increased rate of pressure fluctuations following intravenous injection of noradrenaline (nor), 100 and 200 ng, and angiotensin (ang), 20, 50 and 100 ng.

papilla ; a corresponding rhythmical flow of tubular fluid could also be detected in some of the superficial collecting ducts. The situation of the circular pelvis muscle around the tip of the papilla is an ideal one for causing outflow obstruction, since contraction of the circumference of the papilla would result in a reduction in the radius of all collecting ducts, and hence a marked increase in their resistance to flow.

In the present investigation, angiotensin and adrenaline were found to contract the circular muscle, whereas noradrenaline produced only a weak rise in tone. The actions of the catecholamines appeared to be solely on $\alpha$-adrenoceptors, since they were completely prevented by phentolamine, and since isoprenaline had no stimulant effect on this tissue. Thus the rat renal pelvis muscle is similar to the rabbit uterus (Ahlquist, 1948) in that adrenaline is a more potent stimulant of $\alpha$-adrenoceptors than noradrenaline.

The effect of angiotensin seems to be due to direct stimulation of smooth muscle cells. Nicotine produced no effect on the isolated pelvis muscle, reflecting the absence of ganglion cells from this area (Gruber, 1933 ; Notley, 1969). Tyramine, which is a potent stimulus to the release of noradrenaline from sympathetic post-ganglionic nerve endings, also produced no increase in tone ; thus the contraction produced by angiotensin was unlikely to be due to stimulation of sympathetic ganglia, or to the release of catecholamines.

A constriction of the circular pelvis muscle could explain the raised distal intra-tubular pressure during angiotensin diuresis in the rat (Finberg & Peart, 1970). The variability in responsiveness of the smooth muscle to agonists could explain the fact that this phenomenon was not invariably observed on angiotensin infusion, although variables in setting up the *in vitro* preparation may have caused an apparent range of sensitivity. Slight differences in the anatomy of the pelvis may also contribute to the variability observed.

Contraction of the pelvis muscle could be responsible for the observation by Von Mangos & Braun (1967) that infusion of adrenaline in rats was accompanied by an increased pressure gradient between distal tubule and ureter, indicating an increased resistance to flow in the medullary area of the kidney. The level of urine flow attained during adrenaline infusion (1·5 to 3·0 $\mu$g/min) was not specified, however, and so it is not possible to say whether the effect corresponds to that observed during angiotensin infusion.

Thus although it cannot be proved that an increased pelvis muscle tone could elevate collecting duct flow resistance, yet the available evidence strongly indicates the possibility of such a mechanism. These results also draw attention to the necessity of considering urinary tract contractility when the action of substances with potential smooth muscle stimulant properties is examined in the intact kidney.

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(*Received March* 4, 1970)