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The pharmacology of the rat ureter *in vivo*

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Summary

1. A method of recording the peristaltic frequency and the rate of transport of fluid (perfusion rate) in the rat ureter *in vivo* is described.

2. Acetylcholine and atropine did not alter ureteral activity. Histamine increased the rate of peristalsis by up to 15% and the rate of perfusion by up to 10%. Low doses of 5-hydroxytryptamine increased peristaltic frequency whereas high doses decreased peristaltic frequency; all doses reduced the rate of perfusion.

3. Morphine reduced the rate of perfusion by 5–10% at all dose levels, but only the highest dose used reduced the frequency of ureteral peristalsis.

4. (−)-Adrenaline, (−)-noradrenaline and (±)-isoprenaline reduced the frequency of peristalsis. The order of potency was isoprenaline>noradrenaline> adrenaline. The response was dose-related and blocked by propranolol, which itself did not affect ureteral activity.

Introduction

The response of the ureter to drugs *in vivo* has been investigated in many species, with a variety of results. There are two main reasons for the confusion and contradiction that exists. First, the ureter is a pressure sensitive organ and small changes in pressure in the renal pelvis can affect peristaltic activity in the ureter. Since the rate at which urine is produced by the kidney is readily affected by the autonomic nervous system and by drugs, it seems possible, that when this factor is not taken into account, results represent nothing more than the reaction of the ureter to changing rates of urine production.

Secondly, the smooth muscle of the ureter is particularly susceptible to physical interference. The most commonly used method of recording ureteral activity *in vivo*, the indwelling catheter, has been shown to interfere with the normal functioning of the ureter (Backland, 1963; Weinberg, 1967). In the experiments reported now the effects of drugs on the rat ureter *in vivo* were investigated by a method which records peristaltic activity in the ureter with a minimum of physical interference and eliminates the possibility of ureteral activity being influenced by the rate of urine production. This method has been demonstrated to the British Pharmacological Society (Ancill, Jackson & Redfern, 1970) and some of our results have been reported (Ancill, Jackson & Redfern, 1971).

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Methods

Male Sprague-Dawley rats, weighing 300–350 g, were anaesthetized with pentobarbitone sodium (Nembutal-Abbott), 60 mg/kg i.p. A tracheal cannula was inserted and artificial respiration was carried out at a rate of 72/min with a Palmer miniature respiration pump. A femoral vein was cannulated for the injection of drugs. Arterial blood pressure was recorded from a carotid artery by means of a transducer.

The left ureter, which was the more accessible, was exposed through a midline incision in the abdomen. The abdominal cavity was immediately filled with warm light liquid paraffin and any exposed gut covered with warm saline swabs. To eliminate the influence of urine production by the kidney, the left renal artery and vein were tied and cut. A No. 27G needle was then inserted into the pelvis of the kidney through the renal parenchyma, and the ureter perfused from a reservoir of 0·9% w/v NaCl solution (saline) which contained 0·002% w/v Evan's Blue to aid visual observation. A drip chamber was included in the perfusion system so that the rate of flow could be measured by means of a modified Palmer photoelectric drop recorder. When the needle was in place, the reservoir was raised until the intraluminal pressure was sufficient to induce the passage of small spurts of saline down the ureter. The position of the needle was subsequently verified histologically. To prevent changes in pressure at the distal end of the ureter, a No. 16 needle connected to a length of Portex tubing was inserted into the bladder to act as a drain.

Peristaltic frequency was measured from the action potential associated with each peristaltic wave, recorded extracellularly by means of a glass micro-electrode, with a flexible tip. The micro-electrodes were pulled from soda-glass tubing of 1·0 mm external and 0·75 mm internal diameter to have a shank length of 15–20 mm and a tip diameter of approximately 4 μm. They were filled *in vacuo* with 3 M KCl, and only those having a resistance of less than 10 MΩ were used. The micro-electrode was linked to the pre-amplifier (Tektronix 102, input impedance 10 MΩ) via an agar-KCl bridge and an Ag-AgCl electrode. The potentials obtained from the micro-electrode were amplified differentially with respect to an indifferent electrode which was a hypodermic needle inserted into the left hind limb of the rat.

The micro-electrode was inserted above the region of the ileolumbar vessels where the ureter was free of fat. The electrode was held in a Horsley-Clarke stereotaxic instrument and inserted under direct observation from a binocular microscope.

Drugs

The drugs used were: acetylcholine chloride, histamine dihydrogen phosphate, 5-hydroxytryptamine creatinine sulphate, morphine sulphate, (−)-adrenaline hydrogen tartrate, (−)-noradrenaline hydrogen tartrate, (±)-isoprenaline hydrochloride and (±)-propranolol hydrochloride. All drugs were freshly prepared in saline and were administered intravenously in volumes not exceeding 1 ml. The effects of morphine and the lower doses of atropine (0·03–0·13 μg/kg) were determined by cumulative dosing. All doses are expressed as the weight of base (μg or mg/kg). Student's *t* test was used to assess the statistical significance of the results.

Results

Acetylcholine and atropine. Acetylcholine (0·17–6·7 μg/kg) or atropine (0·03–0·13 μg/kg) did not affect the frequency of ureteral peristalsis or the rate of perfusion in the ureter.

Histamine. The increase in the rate of perfusion (up to 10%) was statistically highly significant at all dose levels (0·3–26·7 μg/kg), while the increase in peristaltic frequency (up to 15%) was significant only at the higher dose levels (6·7–26·7 μg/kg) (Table 1). Although the magnitude of the increase in peristaltic frequency with increased doses of histamine was only slight, the duration of the response increased considerably with dose.

TABLE 1. *The effects of histamine, 5-hydroxytryptamine (5-HT) and morphine on peristaltic frequency and perfusion rate in the rat ureter*

Drug (μg/kg)		Peristaltic frequency (% of pre-injection value)		Perfusion rate (% of pre-injection value)	
Saline		100·7± 1·4	(25)	99·4± 0·7	(15)
Histamine	3·3	107·9± 3·3	(4)	109·1± 3·5†	(4)
	6·7	111·0± 8·7*	(5)	106·8± 2·0†	(4)
	13·3	114·0± 8·5*	(4)	107·7± 2·9†	(5)
	26·7	115·9± 9·2*	(4)	108·8± 4·9†	(4)
5-HT	3·3	113·4±11·6*	(6)	89·9± 5·3*	(5)
	6·7	113·5±11·2*	(6)	85·3± 8·7*	(5)
	13·3	80·6±11·0†	(5)	91·4± 1·4†	(5)
	26·7	82·1± 5·4†	(3)	51·1±12·6†	(5)
Morphine	10	104·9± 4·6	(3)	94·8± 1·1*	(3)
	23	101·4± 9·2	(3)	93·2± 2·6*	(3)
	50	105·7± 8·5	(3)	90·6± 7·4*	(3)
	103	96·8± 5·2	(3)	92·4± 1·6*	(3)
	210	90·0± 7·7*	(3)	92·0± 1·7*	(3)

The values were obtained in the 1-min period beginning 1 min after injection of histamine or morphine, or in the 1-min period immediately after the injection of 5-HT. They are the means ±S.E. expressed in % of the values obtained in the 1-min period immediately before injection. The numbers of observations are given in parentheses. * $P<0·05$, † $P<0·001$, compared with pre-injection values.

TABLE 2. *The effects of noradrenaline, adrenaline and isoprenaline on peristaltic frequency in the rat ureter*

Drug (μg/kg)		Peristaltic frequency (% of pre-injection value)	
Noradrenaline	0·3	100·8±5·4	(4)
	0·7	104·9±5·1	(4)
	1·3	88·6±1·9*	(4)
	2·7	53·3±9·8†	(3)
	3·3	25·2±4·1†	(3)
Adrenaline	1·3	93·7±4·5	(5)
	2·7	89·0±4·2*	(8)
	6·7	82·8±7·2*	(8)
	13·3	73·9±5·5†	(8)
Isoprenaline	0·3	47·9±3·3†	(5)
	0·7	42·4±2·5†	(5)
	1·3	32·4±2·5†	(4)
	2·7	14·4±8·3†	(3)

The values were obtained in the 1-min period beginning immediately after injection of the catecholamines. They are the means±S.E., expressed in % of the values obtained in the 1-min period immediately before injection. The numbers of observations are given in parentheses. * $P<0·05$, † $P<0·001$, compared with the pre-injection values.

5-Hydroxytryptamine. A statistically significant fall in the rate of perfusion was produced at all dose levels (3·3–26·7 μg/kg). Low doses of 5-hydroxytryptamine (3·3–6·7 μg/kg) increased peristaltic frequency, whereas high doses (13·3–26·7 μg/kg) decreased peristaltic frequency (Table 1).

Morphine. Although the rate of perfusion was significantly reduced by 5–10% at all dose levels (10–210 μg/kg), only the highest dose (210 μg/kg) produced any significant reduction in the frequency of peristalsis (Table 1).

Adrenaline, noradrenaline and isoprenaline. Table 2 shows the effects on peristaltic frequency of adrenaline (1·3–13·3 μg/kg), noradrenaline (0·3–3·3 μg/kg) and isoprenaline (0·3–2·7 μg/kg), respectively. All three amines produced a statistically significant reduction in the frequency of peristalsis, with no evidence of initial stimulation.

Propranolol. Administration of propranolol (1·5 mg/kg) did not affect peristaltic frequency. However, in animals to which this dose of propranolol had been administered 10 min previously, adrenaline, noradrenaline and isoprenaline failed to inhibit ureteral peristalsis (Fig 1).

Discussion

The method used in this investigation of ureteral activity *in vivo* avoids in large measure the disadvantages apparent in many previous investigations, in that phy-

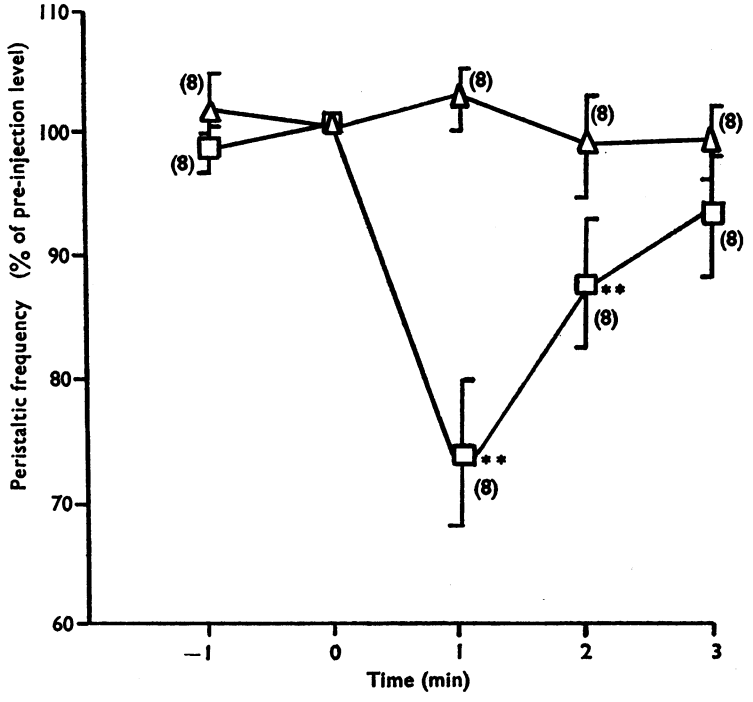

FIG. 1. The effect of intravenous injection of adrenaline, 13·3 μg/kg, at 0 min, on the frequency of peristalsis in the ureter of control animals (□), and of animals pretreated with propranolol, 1·5 mg/kg (△). The number of peristaltic events in successive 1 min-periods before and after injection are expressed in % of values obtained in the 1-min period immediately before injection and is shown with its standard error of the mean. The numbers of observations are shown in parentheses. **, $P<0·001$, compared with pre-injection values.

sical damage to the ureter is minimal and the effects of variation in urine volume are eliminated.

Histamine was found to stimulate ureteral peristalsis up to 15% and increase the rate of perfusion. Thse results are in agreement with those of some workers using both *in vivo* and *in vitro* methods in a variety of species (Boyarsky, 1964; Sharkey, Boyarsky, Catacutan-Laby & Martinez, 1965; Butcher, Sleator, Werner & Schmandt, 1957; Boatman, Lewin, Culp & Flocks, 1967; Kiil & Kjekshus, 1966). Other workers, however, have reported a decrease in the rate of perfusion by histamine (Blackmore, Wilson & Sherrod, 1953; Mazzella & Schroeder, 1960). The most likely explanation of this discrepancy is that the decrease in the rate of flow reported by some workers results from a decrease in the rate of urine production, itself consequent on a fall in blood pressure. When this effect is eliminated, as in our experiments, the effect of a small stimulation of the ureteral smooth muscle by histamine is seen.

5-Hydroxytryptamine causes a reduction in urine flow (Erspamer, 1966) and would therefore be expected to reduce peristaltic frequency in the ureter indirectly. In our experiments where any such indirect effect is prevented, 5-hydroxytryptamine caused an increase in peristaltic frequency at low dose levels, and a decrease at high dose levels. It seems probable that this dual response results from direct stimulation of the smooth muscle of the ureter. An increase in excitability of the smooth muscle was reflected in the increase in the frequency of peristalsis, whereas increased muscle tone decreased the size of the lumen and led to a decrease in the rate of perfusion. There is so far no explanation of the fact that higher doses inhibit the frequency of peristalsis, particularly since there is no evidence of the presence of autonomic nerve plexuses or enteroargentaffine cells in the ureter.

Nelse (1935) reported that morphine increased the amplitude of ureteral contraction in man, while other workers (Lapides, 1948; Kiil, 1957; Weinberg & Maletta, 1963) found that morphine did not have this effect. In our experiments, morphine reduced the rate of perfusion in the ureter by 5–10% over a wide range of doses, but reduced peristaltic frequency only at the highest dose level. The chief interest in these results lies in the fact that morphine may increase the tone of the smooth muscle, a fact that should be taken into consideration in the treatment of renal colic. Morphine may reduce the flow by an increased release of antidiuretic hormone.

In experiments in which the effects on blood pressure and urine flow were not controlled, the catecholamines have been reported to produce a variety of responses in the ureter. For instance, the effect of adrenaline on the dog ureter has been reported to be inhibitory (Mazzella & Schroeder, 1960) excitatory (Greene & Essex, 1942; Kawamata, 1959; Kiil & Kjekshus, 1966; Boatman *et al.*, 1967) or nonexistent (Butcher *et al.*, 1957). Similar conflicting reports can be found regarding the responses to adrenaline, noradrenaline and isoprenaline in a variety of species.

In our view these conflicting results can be explained by a complex combination of direct and indirect responses. In our experiments, in which indirect effects were eliminated, the effect of all three amines was always a reduction in the frequency of peristalsis. The order of potency was isoprenaline>noradrenaline>adrenaline. Since in our experiments, the inhibitory response to all three amines was prevented by propranolol, the amines probably acted on β-adrenoceptors.

Neither we nor any other workers have found effects of acetylcholine on the rat ureter but, in the dog, cholinomimetic drugs have been shown to be excitatory (Greene & Essex, 1942; Kawamata, 1959; Kiil & Kjekshus, 1966; Boatman *et al.*, 1967).

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