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Effect of tetracyclines and 4-epiderivatives on the ureter

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Summary

1. The effect of tetracyclines on the isolated dog ureter is dependent on: (a) the tetracyclines used—mepicycline and doxycycline antagonizing, and tetracycline and rolitetracycline increasing the contractor action of barium chloride; (b) the percentage of 4-epiderivatives in the tetracyclines used—the higher the epiderivative concentration, the smaller the effect of mepicycline or doxycycline, and the greater the action of tetracycline or rolitetracycline.

2. *In vivo* the addition of the antibiotics into the renal pelvis shows no significant differences between the various tetracyclines or different 4-epiderivative concentrations on the intra-ureteral flow of the dog or guinea-pig.

3. Intravenous injection of mepicycline or doxycycline does not induce a significant change in the intra-ureteral flow, while intravenous administration of tetracycline or rolitetracycline produces a triphasic response: (a) a marked decrease of the intra-ureteral flow for a few minutes; (b) a return to the control condition for 30–60 min; and (c) a lesser but persistent decrease in flow for 60–120 minutes. In the first phase the ureteral smooth muscle is directly affected by the antibiotics circulating in the blood, while in the third phase the tetracyclines act via the intra-ureteral mucosa.

4. Neurogenic effects on the ureter-bladder junction *in vivo* are not affected by the tetracyclines tested.

Introduction

Tetracycline, chlortetracycline and oxytetracycline decrease biliary flow in the guinea-pig, cat, dog, and monkey *in vivo*, while their action is rather variable *in vitro*, the most frequent response being a flow decrease. In the isolated gall-bladder, motility is depressed, with or without reduction of tone depending on the concentration. The failure of adrenoceptor, cholinoceptor, histamine, 5-hydroxytryptamine and ganglion blocking agents to prevent the action of the tetracyclines indicates that these antibiotics, as well as rifamycin and erythromycin, act directly on the bile-duct smooth muscle (Benzi, Crema, Bertè & Frigo, 1967).

On the other hand, tetracyclines increase the motility and/or the tone of the dog bronchial chain *in vitro*, the order of potency in the excitatory activity being rolitetracycline>tetracycline>mepicycline$\geqslant$doxycycline. The time/action relationships *in vivo* indicate three distinct phases in tetracycline activity on the respiratory tract: (a) a hyperventilation phase, lasting for a few minutes, (b) a depression of respiratory activity phase, for 60–90 min, and (c) a compensatory phase, with return to the control condition after 90–180 minutes. The intensity of action and the length of the phases are affected by: (a) the tetracycline used, the order of potency being rolitetracycline>tetracycline>doxycycline$\geqslant$mepicycline; (b) the dose used: the

larger the dose, the greater the maximum effect and the longer the overall duration of action ; and (c) the percentage of the 4-epiderivative in the tetracycline used: the higher the epiderivative concentration, the greater the effect (Benzi, Bertè, Arrigoni, Ferrara & Sanguinetti, 1971).

Excretion of the tetracyclines is chiefly in the urine, although only a part of the dose administered is recovered. With tetracycline hydrochloride as much as 25–30% may be recovered, but demethylchlortetracycline is cleared at a much slower rate than other tetracyclines (Kunin & Finland, 1958). The urinary excretion of the tetracyclines opens the possibility of their eventual interference with the tone and motility of the ureteric smooth muscle, as demonstrated previously for ampicillin, dicloxacillin, chloramphenicol, spiramycin and aminosidin.

The purpose of the present report is to evaluate the action *in vitro* and *in vivo* of some tetracyclines on the dog and guinea-pig ureter. The percentage of 4-epiderivative in the preparations used was also determined to assess the extent to which these derivatives could affect the pharmacological response. A Fanconi-like syndrome characterized by nausea, vomiting, acidosis, proteinuria, glycosuria, and aminoaciduria has been associated with degraded tetracycline (Gross, 1963 ; Frimpter, Timpanelli, Eisenmenger, Stein & Ehrlich, 1963 ; Robins, 1963 ; Zimmerman & Werther, 1964 ; Wegienka & Weller, 1964). This syndrome may be a result of the action of the degradation products anhydrotetracycline and epianhydrotetracycline. In rats anhydro-4-epitetracycline induces proteinuria, glycosuria, cellular debris in the urine sediment, and elevated UGOT activity together with necrosis of the convoluted tubules in the renal cortex (Benitz & Diermeier, 1964).

Methods

The following tetracyclines were used (different samples with the indicated percentage of epiderivative): tetracycline (7·4 ; 25·7 ; 39·4), doxycycline (6·8 ; 17·6 ; 37·4), mepicycline (0·8 ; 7·2 ; 18·8 ; 41·2), and rolitetracycline (7·4 ; 30·2 ; 47·7). The separation and determination of the tetracyclines and the 4-epiderivatives was done according to the methods of Lodi, Meinardi & Rossi (1969) and Gyanchandani, McGilveray & Hughes (1970). The concentration of the degradation products was calculated as a percentage of the total tetracycline.

Experiments in vitro

The ureters were taken from ten adult guinea-pigs of either sex, weighing 490–580 g, and from sixteen adult male and female dogs, weighing 6·8–17·4 kg. The ureters were carefully dissected and set up in a 20 ml or 50 ml organ-bath containing Tyrode solution gassed with 95% oxygen and 5% carbon dioxide ; the temperature was 37·0° to 37·5° C. The longitudinal movements of the ureter were recorded by a strain-gauge ; the lever exerted a tension of 0·5 to 1·5 g on the tissue and the magnification was 10 to 20 times.

The action of the tetracyclines was evaluated on the normal ureter or after stimulation by barium chloride. For the construction of the dose-response curves, the tetracyclines activity was taken as the change in the recorded area of barium chloride induced movements during a 30 min period of contact before and after antibiotic addition. Tetracyclines ($11{\cdot}25 \times 10^{-7}$M) were added to the bath 2 min before the barium chloride administration.

Experiments in vivo

Twenty adult female guinea-pigs (415–760 g) and twenty dogs of either sex (6·7–17·4 kg) were used. The guinea-pigs were anaesthetized with urethane (1 g/kg i.p.) and chloralose (20 mg/kg i.p.). The dogs were sedated with urethane (0·4 g/kg i.p.) and anaesthesia was maintained by chloralose (100 mg/kg i.v.) or by nitrous oxide or cyclopropane in closed circuit. The animals were given artificial ventilation. Arterial blood pressure (1 mmHg≡1·333 mbar) was measured from a cannula inserted into a carotid (guinea-pig) or femoral (dog) artery. After laparotomy, a cannula was inserted into the renal pelvis to add Tyrode solution with or without tetracyclines. In guinea-pigs, a draining tube was inserted into the fundus vesicae; in dogs a urethral catheter was inserted. The flow into the ureter was measured for 120 min after the addition of tetracyclines, and compared with the flow during the 60 min period before the addition of the antibiotics. In some animals the ureter was made hypertonic by perfusing barium chloride (50–400 μg/ml) through it.

In twelve dogs the action of the tetracyclines on ureteric flow was evaluated after intravenous injection of 10–20 mg/kg.

In six dogs electrodes were placed around the hypogastric or pelvic nerves. Rectangular pulses, of 1 to 2 ms duration and 5–20 V strength, were applied at a frequency of 5–10 Hz for 20 s, before and after the addition of tetracyclines into the renal pelvis.

Cross-urine-perfusion preparation

The studies were carried out on three groups of three female beagle dogs (11·9–16·8 kg) under the experimental conditions described above. In each group, one donor animal was given 40 mg/kg of tetracycline intravenously, and the changes in urinary flow were evaluated. Another donor dog was given Tyrode solution intravenously. Urine samples were obtained over 4 h by catheterizing and completely emptying the bladder at each collection period. In the recipient dog the urinary flow was measured in both ureters; the control flow was obtained by perfusing both the ureters with the urine collected from the placebo-treated dog. The response was evaluated by perfusing the recipient in a random order: (a) one ureter with the urine from the tetracycline-treated dog; (b) the other ureter with the urine from the placebo-treated dog.

Results

The action of tetracyclines on the isolated dog ureter stimulated by various concentrations of barium chloride is shown in Fig. 1. The Lineweaver-Burk plots show that the antibiotics' activity is affected by: (a) the type of tetracycline—mepicycline and doxycycline antagonizing, and tetracycline and rolitetracycline increasing the contractor action of barium chloride; (b) the percentage of epiderivative in the tetracyclines used—the higher the epiderivative concentration, the smaller the effect of mepicycline or doxycycline, and the greater the action of tetracycline and rolitetracycline.

The *in vitro* effect of tetracyclines upon barium chloride action is not simply due to the chemical interference with barium ions because, at the concentrations used, tetracyclines cannot combine or precipitate as the barium salt or complex. The

action of the antibiotics on normal dog and guinea-pig ureter was similar to that described above, but obviously not so marked as in the presence of barium chloride.

The action *in vivo* of tetracyclines (added to the solution flowing through the ureter) on ureteral flow is summarized in Fig. 2. No significant differences between the various tetracyclines or the different 4-epiderivative concentrations are evident because of the high variation in response. This behaviour was present even when the tone of the ureter was locally increased with barium chloride.

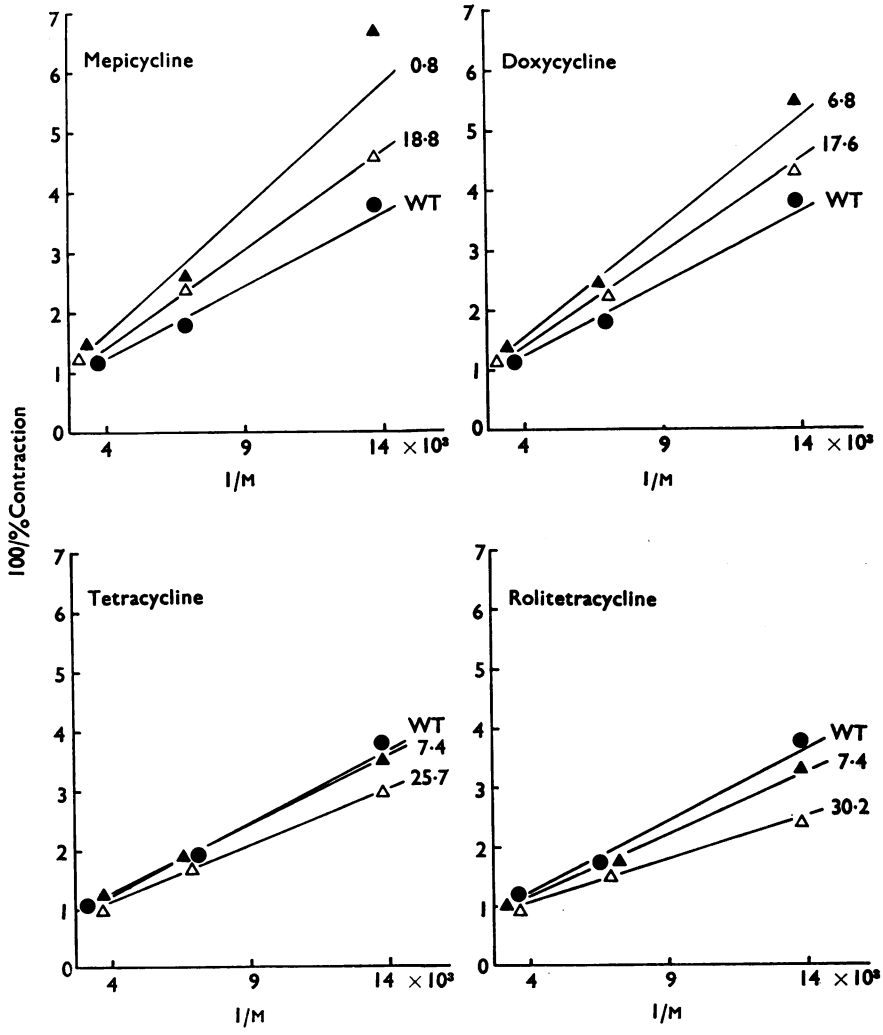

FIG. 1. Dog ureter *in vitro*. Lineweaver-Burk plots calculated: (a) for the effect of barium chloride in the absence of tetracycline, 30 min contact (WT); and (b) for the effect of various concentrations of 4-epiderivatives contained in a constant concentration ($11{\cdot}25 \times 10^{-7}$M) of the tested tetracyclines introduced to the bath 2 min before the barium chloride doses. Abscissa, the reciprocals of barium chloride molar concentration (1/M). Ordinate, the reciprocals of effect (100% contraction). The number by the side of each line indicates the 4-epiderivative concentration. Plotted points represent average values in four preparations for tetracyclines plus barium chloride, and in sixteen preparations for barium chloride alone.

The intravenous injection of mepicycline or doxycycline (10–20 mg/kg) does not induce significant changes in the intra-ureteral flow, while the intravenous administration of tetracycline or rolitetracycline (10–20 mg/kg) produces a triphasic response: (a) marked decrease (range: 30–80%) of the intra-ureteral flow occurs at first for a few minutes, with time to peak effect of 2–4 min; (b) after 6–8 min the flow returns to the control condition; and (c) after 30–60 min a lesser but persistent decrease in flow (range: 5–20%) in the ureter can occur in about 75% of treated dogs.

The cross-urine-perfusion technique, set up to elucidate the mechanism of this behaviour, indicates that in the first phase the ureteral smooth muscle was directly affected by the tetracyclines circulating in the blood, while in the third phase the antibiotics act via the intra-ureteral mucosa. An example is shown in Fig. 3. In the donor dog (treated with tetracycline intravenously) all three phases occurred, while in the recipient dog (perfused into the renal pelvis with the urine from the donor dog) only the third phase was present.

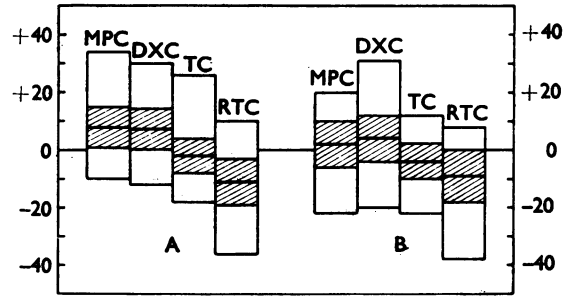

FIG. 2. Action of tetracyclines (added to the solution flowing through the ureter: 500 μg/ml) on dog intra-ureteral flow *in vivo*. Ordinate, mean percentage changes from preaddition values. MPC=mepicycline, with 7·2% of epi-MPC in A, and 41·2% of epi-MPC in B. DXC=doxycycline, with 6·8% of epi-DXC in A, and 37·4% of epi-DXC in B. TC=tetracycline, with 7·4% of epi-TC in A, and 39·4% of epi-TC in B. RTC=rolitetracycline, with 7·4% of epi-RTC in A, and 47·7% of epi-RTC in B. Each column represents the highest and smallest value, the mean value (marked line), and the standard error of the mean (hatched area); $n=5$.

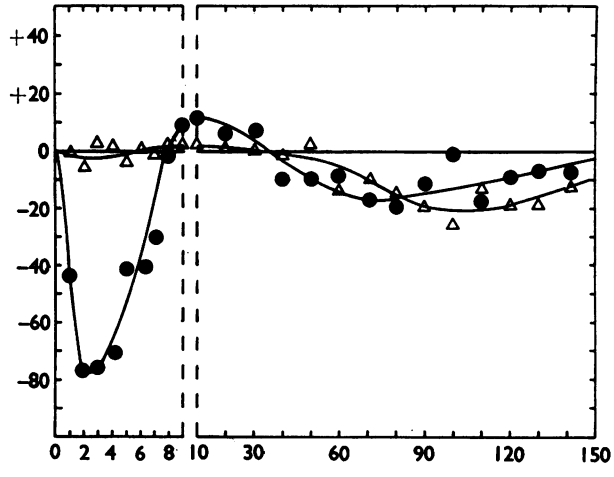

FIG. 3. Cross-urine-perfusion preparation in beagle dog. ●, Donor dog; △, Recipient dog. Ordinate, percentage change of the intra-ureteral flow after the injection of tetracycline (40 mg/kg i.v.) in donor dog, or after the addition of the donor's urine to the renal pelvis of the recipient dog. Abscissa, time (min).

The *in vivo* addition of tetracyclines into the solution flowing through the ureter induced no action on the electrical stimulation of the hypogastric or pelvic nerves, because of the predominantly contractor action of the bladder muscle on the terminal ureteric musculature, which was unaffected by the antibiotics flowing from the ureter-bladder junction into the urinary bladder.

Discussion

The *in vitro* results on the dog ureter confirm the previous observation (Benzi, *et al.*, 1971) that the action of the tetracyclines is dependent on the antibiotic used and its 4-epiderivative concentration. Mepicycline and doxycycline antagonize, while tetracycline and rolitetracycline increase the contractor action of barium chloride on ureteral smooth muscle. Furthermore, the higher the 4-epiderivative concentration, the smaller the effect of the first two antibiotics, and the greater the action of the last two.

In vivo no significant differences were found between the tetracyclines tested while flowing through the ureter. Only the intravenous injection of tetracycline and rolitetracycline produced a marked but transient decrease in the intra-ureteral flow, followed by a return to normal conditions and with a subsequent lesser but persistent decrease in flow.

The initial contracting phase on the ureteral smooth muscle is related to a direct action of the antibiotic passing from the bloodstream to the tissues. The subsequent lesser spasmogenic phase is probably related to passage of the antibiotic across the ureteral mucosa. The intra-ureteral perfusion with tetracyclines has no influence on the neurogenic effects at the ureter-bladder junction induced by electrical stimulation of hypogastric or pelvic nerves, because of the predominating effect of the bladder musculature which is unaffected by the antibiotics flowing from the ureter into the bladder.

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