EFFECT OF ANTIBIOTICS ON THE URETER MOTOR ACTIVITY

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Abstract—The effect of certain antibiotics on the ureteral tone and motility has been studied in vitro and in situ in guinea-pigs and dogs. Tetracycline and rolitetracycline induced a constriction, while ampicillin, isoxazolyl penicillins, gentamicin, aminosidin, spiramycin and chloramphenicol induced a relaxation of the ureter. In vivo, the responses were independent of both bladder activity and systemic effects related to the antibiotic flowing into the ureter and eventually absorbed from it. The antibiotics acted directly on the ureteral smooth muscle; factors influencing the action were: (a) the free or conjugated form of the antibiotic flowing through the ureter; (b) pH of the urine; (c) time of contact; (d) time lag.

Antibiotics have been used extensively in the treatment of urinary tract infections, the kidneys being the main route by which a great number of antibiotics are excreted from the body in both modified and unchanged forms. It is rather surprising that little advancement has been made in the knowledge of the action of the excreted antibiotics on tone and motility of the urinary tract musculature, particularly of the ureter. On the other hand, it is known that antibiotics can interfere with the smooth muscle of the intestinal tract (1-5), of the bronchial tract (2, 6), of the uterus (7), of the extrahepatic biliary tract (8). This report presents experimental findings on the action of certain antibiotics on the tone and motility of the ureter both in vitro and in situ, in guinea-pigs and dogs.

MATERIALS AND METHODS

The experiments were performed in the guinea-pig and in the dog both in vitro and in situ. The antibiotics used were: D(−) chloramphenicol, gentamicin sulphate, aminosidin sulphate, spiramycin adipate (the amounts of which are expressed in terms of the base); ampicillin sodium (the amounts of which are expressed in terms of D(−)-6-(α-amino-α-phenylacetamido)-penicillanic acid); oxacillin sodium, cloxacillin sodium, dicloxacillin sodium (the amounts of which are expressed in terms of 3-phenyl-5-methyl-4-isoxazolylpenicillanic acid). The experiments were performed also with the following tetracyclines (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 amounts of which are expressed in terms of the base. The separation and determination of the tetracyclines and the 4-epiderivatives were made according to the methods of Lodi et al. (9) and Gyanchandani et al. (10). The concentration of the degradation products was calculated as a percentage of the total tetracycline.
Experiments in vitro

Ureters were removed from 40 adult guinea-pigs of both sexes and from 62 adult male and female mongrel dogs. These 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; temp. was $37.2\pm0.2^\circ C$ and pH was buffered to $7.2\pm0.05$. The longitudinal movements of the ureter were recorded by a strain-gauge with isotonic transducer; the lever exerted a resting tension of 0.5 to 1.5 g on the tissue and the magnification was 10-20 times.

The action of the antibiotics was evaluated on the ureter in normal condition or after stimulation by barium chloride (10 to 50 $\mu g/ml$ plus 0.1 $\mu g/ml$ of atropine sulphate), histamine (2.5 to 10.0 $\mu g/ml$) or serotonin (2.5 to 10.0 $\mu g/ml$). For the construction of the dose-response curves, the activity of the antibiotics was taken as the change in the recorded area of barium chloride induced movements during a 30 min period (tetracyclines) or 20 min period (other antibiotics) of contact before and after antibiotic addition. The tested antibiotics were added to the bath 2 min (tetracyclines) or 10 min (other antibiotics) before the barium chloride administration. The ED$_{50}$ and the slope function of the lines, with 95% confidence limits, were evaluated according to Litchfield and Wilcoxon (11).

Experiments in vivo

Studies were performed on 260 adult female guinea-pigs and 56 mongrel dogs of both sexes. 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 induced 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 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 perfuse the ureter by Tyrode solution with and without antibiotics. The pH of the Tyrode solution was generally adjusted to $6.6\pm0.05$, but in some experiments the pH was buffered to $7.8\pm0.05$ or $5.5\pm0.05$. In guinea-pigs, a draining tube was inserted into the fundus vesicae; in dogs, an urethral catheter was inserted. The flow into the ureters was measured for a 30 min period immediately after the beginning of the change induced by antibiotics, compared with the flow during the 30 min period before the addition of the antibiotics. The ureter was either normal or activated by perfusing barium chloride (50 to 400 $\mu g/ml$). The ED$_{50}$ and the slope function of the lines, with 95% confidence limits, were evaluated according to Litchfield and Wilcoxon (11).

In some experiments, prior to addition of an antibiotic to the solution flowing through the ureter, the animals were pretreated with: atropine sulphate (1-2 mg/kg s.c.), chlorpheniramine maleate (1-2 mg/kg s.c.), methysergide maleate (25-50 $\mu g/kg$ s.c.), cyproheptadine hydrochloride (150-300 $\mu g/kg$ i.v.), dibenamine hydrochloride (2-4 $\mu g/kg$ i.v.), $\Delta(-)$ INPEA (4-8 $\mu g/kg$ i.v.), or hexamethonium bromide (100-200 $\mu g/kg$ i.v.).

In a series of experiments it was possible to evaluate in situ the flow through the ureter pulled out from the ureter-bladder junction and therefore free from urinary vesica inter-
In 22 mongrel dogs electrodes were placed around the hypogastric or pelvic nerves. Rectangular pulses, of 0.2-1 msec duration and 5-20 V strength were applied at a frequency of 5 to 10 shocks/sec for 20 sec, before and after the addition of the antibiotics into the renal pelvis.

Cross-urine-perfusion preparation

Studies were carried out on 8 groups of 3 beagle bitches. In each group, one donor animal was given the tested antibiotic i.v.; another donor was given Tyrode solution i.v. During 4-8 hr, urine samples were obtained by catheterizing and completely emptying the bladder at each collection period. In the recipient 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 animal. The response was evaluated by perfusing the recipient in a random order: (a) one ureter with the urine from the antibiotic-treated animal; (b) the other ureter with the urine from the placebo-treated animal.

Linear Graphical Representations

Dixon procedure: This method was applied to study the extent to which the 4-epiderivatives concentrations could affect the pharmacodynamic response induced by tetracyclines. In the Dixon procedure (Dixon 12), the reciprocal of the effect is expressed as a function of the combined drug B concentration, at two fixed concentrations of the tested drug A. In our procedures we expressed the reciprocal of the effect on the ureteral musculature as a function of the 4-epiderivatives concentration (contained in a constant concentration of the tested tetracycline) at two fixed concentrations of barium chloride.

Lineweaver-Burk procedure: This method was used to evaluate the effect of a constant concentration of the tested tetracyclines on the ureter stimulated in vitro with varying concentrations of barium chloride. Quantitative relationships between drug A and receptor:

$$\frac{E_A}{[A]} = \frac{E_{A,max}}{K_A + [A]}$$

where: $E_A =$ pharmacodynamic effect;

$K_A =$ dissociation constant of drug-receptor complex;

$[A] =$ antibiotic concentration

can be rearranged (Lineweaver-Burk, 13) as:

$$\frac{1}{E_A} = \frac{1}{E_{A,max}} + \frac{K_A}{E_{A,max} [A]}$$

The reciprocal of the pharmacodynamic effect is expressed as a function of the reciprocal of the drug concentration. A similar procedure was applied in the case of the interaction between barium chloride and the members of a homologous series of tetracyclines.

RESULTS

1) Pharmacodynamic action of the antibiotics on ureter

Studies in guinea-pigs and dogs demonstrated that chloramphenicol, gentamicin, aminosidin, ampicillin, isoxazolyl penicillins and spiramycin induce both an inhibition in vitro of the spasm by agonist (barium chloride, histamine, or serotonin) and an increase in situ of the flow through the ureter, either normal or activated by agonist. The $ED_{50}$
on the ureter, normal or activated by barium chloride, is summarized in Table 1; the order of potency in the myolytic activity was chloramphenicol, >isoxazolyl penicillins, >gentamicin, >aminosidin, >spiramycin, >ampicillin. As concerns the tetracyclines, their action was affected: (a) by the antibiotic used: mepicycline antagonized, while tetracycline and rolitetracycline increased the contracting action of barium chloride on ureter smooth muscle (Fig. 1); (b) by the percentage of the 4-epiderivatives in the tetracycline used: the higher the 4-epiderivative concentration, the greater the stimulating action of barium chloride (Fig. 2). On the contrary, there were no significant differences among activities of tetracycline, oxytetracycline, and chlortetracycline (Table 2).

The in vivo addition of antibiotics into the solution flowing through the ureter induced no effect on the response to electrical stimulation of the hypogastric or pelvic nerves, due to the predominant contraction of the bladder muscle which was unaffected by the antibiotics flowing from ureter-bladder junction into the urinary bladder.
FIG. 1. Dog ureter in vitro. Lineweaver-Burk plots calculated: (a) for the effect of barium chloride in the absence of tetracycline (WT); and (b) for the effect of a constant concentration ($11.25 \times 10^{-7}$ M) of various tetracyclines ($\blacktriangle$ mepicycline; • tetracycline; □ rolitetracycline) introduced into the bath 2 min before the barium chloride. Abscissa, the reciprocals of barium chloride molar concentration (1/M). Ordinate, the reciprocals of the effect (100/percent of maximal contraction assumed equal to 100). Plotted points represent average values in four preparations for tetracyclines plus barium chloride, and in sixteen preparations for barium chloride alone. The concentrations of 4-epiderivatives contained in the various tetracyclines were the following: mepicycline = 18.8%; tetracycline = 25.7%; rolitetracycline = 30.2%.

FIG. 2. Dog ureter in vitro. Dixon straight lines calculated for the effect of two fixed concentrations of barium chloride ($A_1 = 7.22 \times 10^{-5}$ M; $A_2 = 2.88 \times 10^{-7}$ M) expressed as a function of the 4-epiderivatives concentration contained in a constant concentration ($11.25 \times 10^{-7}$ M) of the tetracyclines tested, introduced into the bath 2 min before the barium chloride. Abscissa, % concentration of the 4-epiderivatives. Ordinate, the reciprocals of the effect (100/percent contraction). Plotted points represent average values in four preparations.
TABLE 2. Dog isolated ureter. ED<sub>50</sub> and slope function of the line (S), with 95% confidence limits, of three different tetracyclines, the 4-epiderivative concentration being quite equal in the preparations (from 23.2 to 29.4%).

| Tetracycline      | ED<sub>50</sub>(a) (µg/ml) | S(b)     | P       |
|-------------------|---------------------------|----------|---------|
| Tetracycline      | 1200                      | 1.74     |         |
| (698 ; 2064)      | (0.97 ; 3.13)             |          |         |
| Oxytetracycline   | 1030                      | 1.62     | >0.05   |
| (536 ; 1978)      | (0.86 ; 3.05)             |          |         |
| Chlortetracycline | 1440                      | 1.88     | >0.05   |
| (600 ; 3456)      | (0.82 ; 4.32)             |          |         |

(a) ED<sub>50</sub>—antibiotic concentration (µg/ml) reducing by 50% the intra-ureteral flow during 30 min period of contact.
(b) S—as in Table 1.

2) Mode of pharmacodynamic action of antibiotic on the ureter

Responses in situ of the ureter to antibiotics flowing through the ureter were independent of the ureter-bladder junction activity, as they were present even when the ureter was freed from urinary musculature. The action was also independent of any systemic effects related to the antibiotic eventually absorbed from ureter into the bloodstream and distributed throughout the body, as the urinary flow into the other ureter was unaffected during the antibiotic action on the ureter being tested.

The direct action of the antibiotics on the extrarenal urinary tract smooth muscle was supported by: (a) the failure of cholinoreceptor, adrenoceptor, histamine, 5-hydroxytryptamine and ganglion blocking agents to prevent the in vivo action of tetracycline and rolitetracycline, (b) the ability of gentamicin, ampicillin, isoxazolyl penicillins, aminosidin, and chloramphenicol to remove in situ the ureteral spasm caused locally by barium chloride, histamine and serotonin.

3) Factors influencing antibiotic pharmacodynamic action

3.1) Free or conjugated form of antibiotics into the urine

This factor has been exemplified by the activity of chloramphenicol and its metabolites on the extrarenal urinary smooth muscle by the cross-urine preparation in the dog. In fact, in a variety of animal species, including man, about 90% of an administered dose of chloramphenicol can be recovered from the urine in 24 hr principally in the form of inactive metabolic products which retain the arylnitrogroup intact. Less than 10% is free active chloramphenicol, 1% represents drug in which the aromatic nitrogroup has been reduced to an aminogroup, and the greater portion of the excreted antibiotic is a therapeutically inactive conjugation product with glucuronic acid (14).

In the present study, the action of the urine from chloramphenicol-treated dogs was similar to the action of the urine from placebo-treated dogs added to the antibiotic at a concentration equal to the microbiological activity of the urine from the chloramphenicol-treated dogs. Assuming that less than 10% of the administered dose is excreted unchanged (as determined by microbiological assay) and that about 90% is excreted
unchanged and conjugated with glucuronic acid (as determined by chemical assay for total nitro compounds), it is possible to conclude that the conjugated antibiotic does not produce any effect on the ureter smooth muscle, the binding of antibiotic with glucuronic acid probably preventing the penetration of the drug to its muscular sites of action.

3.2) The pH of the urine

The pharmacodynamic action of the antibiotics can be pH-dependent, as indicated in Fig. 3 for ampicillin, dicloxacillin, and mepicycline. The ED\textsubscript{50} of both ampicillin and dicloxacillin decreased with the decrease of the pH, while the ED\textsubscript{50} of mepicycline increased with the decrease of the urinary pH. The explanation is that the pH influences both the drug and the receptor. Actually, the antibiotics having the character of bases (as mepicycline) or acids (as ampicillin), the degree of passage across the urinary mucosa is due to the ratio unionized/ionized molecules. No active transport is assumed for ionized molecules. Therefore the extent of penetration into the biophase is greatly affected by the pH of the urine.

In this way an organic acid such as ampicillin passed readily from the acid urine to biophase as it was scantily ionized in this low-pH environment. Conversely, a basic substance such as mepicycline, passed poorly across the mucosa with regard to its high ionization under the same conditions, its passage to biophase being greater from the alkaline urine.

On the other hand, presuming that electrostatic forces are implicated in the "antibiotic-site of action" binding, the interaction can be influenced to a considerable extent by the pH of the biophase in which the basic or acid antibiotics are dissolved and in which lies the contact with the acid or basic target sites. Thus in the biophase the antibiotic and the site of binding could both be influenced by the pH of the biophase itself. Therefore it was possible to suppose that ampicillin, after distribution in the biophase, was pharmacodynamically active in the ionized form. If electrostatic attraction is the force for the interaction with the target site, this must contain a basic grouping and the interaction will depend on the degree of ionization. The lower the pH, the more the cationic groups of the target site attract ampicillin. On the contrary, for mepicycline, it can be presumed that the site of action must contain an acidic grouping and the lower the pH, the less anionic the groups will be, and the less they will be able to attract the antibiotic, as indicated for other drugs by Segre (15), Rocha and Silva (16), and Ariëns and Simonis (17).
3.3) Time lag

As indicated in Fig. 4, there were delay situations in the onset of antibiotic pharmacodynamic action after addition into the urine of what has been evaluated to be an effective dose of the drug. The time for onset of action was concentration-dependent; the greater the concentration, the lower the time lag. The reasons for delays in the onset of effect of antibiotic on the ureter include the time it takes for the passage across the ureteral mucosa, the time for diffusion into the reactive tissues, and the time it takes for the pharmacodynamic effects to reach an evaluable level.

3.4) Time of contact

There is less general uniformity in the time-action relationships than in those of dose-response, so that the formulation of a general description of the phenomenon for antibiotics also has been difficult to develop. Nevertheless it is possible to exemplify for gentamicin and aminosidin that the action on the ureter was a function of the time of contact, as indicated in Fig. 5.

DISCUSSION

Not all pharmacodynamic effects produced by antibiotics in lower-animal experimentation can be extrapolated to humans. In any case, it seems useful to remark that the pharmacodynamic concentrations of antibiotics in extrarenal urinary tract of animals are mostly included within the range of the urinary levels occurring in man after therapeutie doses of antibiotics. For example, the urinary active concentration of aminosidin
on guinea-pig's ureter in situ ranges from 100 to 1600 μg/ml; on the other hand, in man the peak level of the antibiotic varies from 1300 to 2100 μg/ml in the 4 hr urine fractions collected after i.m. administration of a therapeutic dose of 500 mg of aminosidin (18). Likewise, chloramphenicol pharmacodynamic intraureteral active concentrations on normal or activated ureter in situ of guinea-pig range from 4.0 to 45.5 μg/ml; after oral administration of 1.5 g of antibiotic to humans the urinary concentration varies from 70 to 210 μg/ml in 24 hr urine specimens by microbiological assay (14). Therefore, the present experimental data can be used to predict in humans qualitative and quantitative effects that may be expected from an antibiotic, although there is no set of rules of different distribution and elimination rates, and of different rates of antibiotic metabolism.

In any case it is possible to predict the difficulty of the studies in humans because the response of the urinary smooth muscle is dependent on the urinary levels of the antibiotic used, on the time lag and time of contact, on the presence in the urine in free or conjugated form, etc. Evaluations on humans are even more difficult as the time lag, the pharmacodynamic effect etc. are a function of the urinary level, and this parameter changes during the antibiotic urinary elimination as a function of time.

The finding that the pharmacodynamic response to the antibiotics is pH-dependent is particularly important during inflammatory processes of the urinary tract which induce changes of the physicochemical properties of the urine and of the ureteral mucosa with consequent modification of the passage of antibiotics across the mucosa.

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