ACTION OF GENTAMICIN, AMINOSIDIN, AND AMPICILLIN ON UTERINE TONE AND MOTILITY

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The action of the antibiotics on the uterus has been mainly investigated to determine the degree of their efficacy in the treatment of the local infections, with little attention to the possibility of their eventual interference with the uterus tone and motility. On the other hand, it is well known that the antibiotics can interfere with the smooth muscle of the extra-hepatic biliary tract (1, 2), the extra-renal urinary tract (3), the bronchial tract (4) and the intestinal tract (5-9). Concerning the influence of some antibiotics on uterine motor activity, Popovici et al. (10) found in the guinea-pig that Erythromycin, Streptomycin, Chlortetracycline, and Chloramphenicol had a stimulating action. While with Erythromycin only this effect was seen, independent of the dose used. Inhibitory action, in larger doses, constantly appears after use of Streptomycin and very seldom after Chlortetracycline and Chloramphenicol. The mechanism producing these effects is different in pregnant and non-pregnant states.

In the present paper the action of Gentamicin, Aminosidin and Ampicillin on the uterine tone and motility of guinea-pig and dog, both in vitro and in situ, is described.

MATERIALS AND METHODS

1) Design of experiment: Studies were performed both in vitro on uterine tissue from guinea-pig, and in situ on uteri of guinea-pig and beagle dog. Each antibiotic was studied in adult non-pregnant and pregnant animals; in some cases the physiologic state of the uterus was influenced by the treatment with chorionic gonadotropin and estradiol, according to Porter et al. (11). The animals were sometimes “sensitized” by subcutaneous injection with diethylstilbestrol in oil 1 or 2 days prior to the experiment according to the method of Lish et al. (12).

2) Drugs used: Gentamicin sulphate (Essex Italia), Aminosidin sulphate (Farmitalia), Papaverine hydrochloride (Carlo Erba), the amounts of each expressed in terms of the base; Ampicillin sodium (Farmitalia), the amounts expressed as D(-)-6-(α-amino-α-phenylacetylamo)-penicillanic acid.

3) Operative procedure

3. 1) Experiments in vitro: Female guinea-pigs weighing 260 to 310 g were used. Immediately after sacrificing and bleeding, the right horn of the uterus was removed and set up in a 20 ml isolated organ bath filled with Krebs-Henseleit or Tyrode solution maintained at 37.5°C and aerated with bubbling oxygen. The horns were suspended vertically
from an optimally loaded isotonic lever (13), with the vaginal part at the bottom, the lever being fixed to a strain-gauge connected to an electronic microdynamometer. Transmural stimulation was carried out by inserting a silver wire into the lumen of horn and placing the reference electrode in the bath. During electrical stimulation of the uterus, spontaneous activity was abolished by lowering the temperature of the bath to 28°C (14). Optimal stimuli from a square wave electronic stimulator were determined for each preparation (12, 13). Uterine muscle contraction during control trials was compared to that performed following a 10 min period of exposure to the test compounds.

ED$_{50}$ and slope function of the line, with 95% confidence limits, were calculated, according to Litchfield and Wilcoxon (15).

3. 2) Experiments in situ: In a first series of experiments on guinea-pig (weighing from 320 to 390 g) isotonic tonus and contractions of uterus were recorded using an optimally loaded lever attached to the uterine horn. The lever was fixed to a strain-gauge connected to an electronic microdynamometer. To analyse the mode of action, the antibiotics were assayed after treatment with the following drugs: Atropine sulphate (BDH): 2-3 mg/kg s.c.; Dibenamine hydrochloride (Prodotti Gianni): 3-6 mg/kg i.v.; INPEA (Bioterapico Selvi): 4-8 mg/kg i.v.; Methysergide maleate (Sandoz): 20-40 μg/kg s.c. +10 μg/kg i.v.; Chlorpheniramine maleate (Essex Italia): 2-4 mg/kg s.c.; Cyproheptadine hydrochloride (Merck Sharp & Dohme-Italia): 200-400 μg/kg i.v.; Hexamethonium iodide (schuchardt): 200-400 μg/kg i.v..

In a second series of experiments on beagle dogs (weighing 9.8 to 13.2 kg) intraluminal pressure was measured from the cervical tip, using an open-tipped saline- or oil-filled polyethylene catheter inserted through the vagina and connected to a pressure transducer. According to Lish et al. (12), isometric tension of the longitudinal muscle of the same horn was measured with a strain-gauge force displacement transducer. Both the resulting pressure- and force-induced electrical analogues, and the arterial blood pressure were recorded on a Schwarzer’s 12-channel Physioscript recorder.

The tested antibiotics were injected intravenously by a cannula inserted into the jugular vein (guinea-pig) or into the femoral vein (dog).

RESULTS
1) Action of antibiotics on the uterus in vitro

As indicated in Table 1, Gentamicin induced an excitatory effect manifested by enhancement of the tonus level and an increase in the frequency of contractions until the disappearance of all spontaneous movements, as a result of hypertonus. This was without any particular difference between pregnant and non-pregnant animals. The stimulating effect of Gentamicin was antagonized by Papaverine (Fig. 1).

Aminosidin and Ampicilin induced an inhibitory effect manifested by a decrease of both the tonus level and frequency of the contractions, with an increase in the height of spontaneous movements, as indicated in Table 1. No differences were observed between uteri removed from pregnant or non-pregnant adult guinea-pigs. Aminosidin and Ampicil-
TABLE 1. In vitro action of Gentamicin, Aminosidin and Ampicillin on the uterus of both pregnant and non-pregnant adult guinea-pig.

| Antibiotics | Concentration (g/ml) | n | Uterine activity |
|-------------|----------------------|---|------------------|
| Gentamicin  | 2.5 × 10⁻⁹ to 1 × 10⁻⁵ | 14 | slight increase  |
|             |                     |   | slight increase  |
|             | to 2.5 × 10⁻⁶       | 20| moderate increase|
|             |                     |   | increase decrease|
|             | to 1 × 10⁻⁷         | 13| marked increase  |
|             |                     |   | abolished because of the hypertonus|
| Aminosidin  | 3.2 × 10⁻⁴ to 3.2 × 10⁻² | 22| normal           |
|             |                     |   | marked decrease  |
|             | 1.3 × 10⁻⁵          | 21| marked decrease  |
|             |                     |   | moderate decrease|
|             | 5 × 10⁻⁶            | 18| normal or slight decrease |
|             | 4 × 10⁻⁸            |   | slight decrease  |
|             |                     |   | moderate increase|
| Ampicillin  | 4 × 10⁻⁶ to 3.2 × 10⁻⁴ | 21| moderate decrease|
|             |                     |   | moderate decrease|
|             | 1.3 × 10⁻³          | 17| considerable decrease |
|             |                     |   | marked decrease  |

Fig. 1. Guinea-pig uterus “in vitro”. Lineweaver-Burk plots calculated for the combinations of varying concentrations of Gentamicin (20 min of contact) with two constant concentrations of Papaverine (1.17 and 2.35 × 10⁻⁵ M) introduced to the bath 5 min before Gentamicin doses.

Abscissa: the reciprocals of Gentamicin molar concentration (1/M).
Ordinate: the reciprocals of effect (100% contraction by Gentamicin).
Plotted points represent average values of 6 preparations.
lin inhibited contraction of the sensitized, optimally loaded, isolated uterus of guinea-pig, contracting isotonically in response to optimal electrical transmural stimulation. The ED₉₀ of the two antibiotics and Papaverine is summarized in Table 2.

2) Action of antibiotics on the uterus in vivo

As indicated in Table 3, Gentamicin induced a stimulating effect, while both Aminosidin and Ampicillin induced an inhibitory effect on the uterine activity of adult guinea-

| Drug       | ED₉₀ (µg/ml) | (b) S          |
|------------|--------------|---------------|
| Ampicillin | 620 (248–1550) | 3.20 (0.71–14.40) |
| Aminosidin | 900 (407–1989) | 2.29 (0.65–8.01)  |
| Papaverine | 4.8 (2.0–11.0) | 2.81 (0.85–9.27)   |

(a) ED₉₀ = drug concentration (µg/ml) reducing by 50% the response of the transmural stimulated uterus.

(b) S = fold change in dose required to produce a unit standard deviation change in response along the line; thus S = antilog s = antilog 1/b, where b and s are respectively the slope constant and standard deviation of a line relating log dose of drug, and probit per cent effect (Litchfield and Wilcoxon, 15).

| Antibiotics  | Doses mg/kg i.v. | Animals | n | Tone  | Frequency of contractions | Height of contraction | Duration of contraction |
|--------------|------------------|---------|---|-------|---------------------------|----------------------|------------------------|
| Gentamicin   | 0.5–2.5          | non-pregnant | 13 | slight increase | normal      | normal                 | normal                 |
|              |                  | pregnant   | 12 | moderate increase | slight increase | normal                 | normal                 |
|              | 2.5–20           | non-pregnant | 9  | moderate increase | moderate increase | moderate increase or abolished |                      |
|              |                  | pregnant   | 16 | marked increase | moderate increase | decreased or abolished due to hypertonus |                      |
| Aminosidin   | 2.5–20           | non-pregnant | 9  | normal        |               | moderate increase       |                       |
|              |                  | pregnant   | 14 | normal or moderate decrease | moderate decrease |                         |                       |
| Ampicillin   | 2.5–20           | non-pregnant | 10 | normal        |               | normal or slight increase |                       |
|              |                  | pregnant   | 14 | normal or moderate decrease | moderate decrease |                         |                       |
pig. There were no significant differences in the responses of anestrus guinea-pigs, "sensitized" guinea-pigs and estrus guinea-pigs to the uterine action of the tested antibiotics. The estrogen-dominated uteri usually showed greater spontaneous movements than progesterone-dominated uteri, but a non-significant difference in the effect of the tested antibiotics.

The antibiotics exerted in the beagle dog similar effects to that in guinea-pig when given in the same doses. Investigation on the action of Gentamicin, Aminosidin and Ampicillin as to the correlation between changes in isometric tension of the longitudinal muscle and the intraluminal pressure changes related to circular muscle, indicated that the antibiotics did not exclusively effect one or the other. Furthermore, no consistent differences in the effect of antibiotics on the pressures recorded from the fundic and cervical areas were seen.

In uterus response to antibiotics no modifications were induced by the pretreatment with Atropine, Chlorpheniramine, Methysergide, Cyproheptadine, Dibenamine, INPEA, and Hexamethonium. This behaviour confirmed the observations of Popovici et al. (10) that uterine responses to antibiotics are the consequence of direct action on uterine muscle.

3) Prediction for clinical pharmacology and therapeutics

Even though extrapolations for humans are problematic and doubtful, it should be beneficial for clinical studies to remark that doses/kg of the tested antibiotics effective on the uterine activity of non-pregnant and pregnant guinea-pigs and dogs were for the greater part included within the range of doses used for humans during therapeutical treatment of obstetrical and gynecological infections.

The different response of the uterus to the various antibiotics indicates that the clinical choice of antibiotic for the treatment of the inflammatory processes of the female reproductive system could be made almost dependent on: 1) antibacterial spectrum; 2) local active concentration; and 3) action on the uterine muscle.

DISCUSSION

Gentamicin induced on the pregnant and non-pregnant uterus a stimulating effect manifested by a raise of the tonus and an increase in the waves frequency, until the disappearance of spontaneous motor activity due to hypertonus. Conversely, Aminosidin and Ampicillin depressed the tonus level and the frequency of the waves of the uterus whether normal or stimulated with the height of the waves being increased.

There were no observed significant differences between the actions of the tested antibiotics on anestrus, estrus, and sensitized animals, or on estrogen- and progesterone-dominated uteri. The antibiotics did not exclusively effect circular and longitudinal uterine muscle, or fundic and cervical areas.

The behaviour of the tested antibiotics to the pretreatment with various potential antagonists confirmed the conclusions of Popovici et al. (10) that antibiotics induce a direct action on uterine musculature.

It is important to draw attention to the fact that the in vivo doses of antibiotics able to induce an action on the uterus were generally included within the range of doses employed
for human therapeutical treatment.

In conclusion, it could be beneficial for clinical pharmacology and therapeutics to choose an antibiotic on the basis not only of antibacterial spectrum and local active concentration, but also as to specific action on uterine muscle.

SUMMARY

Influence in vitro and in vivo of Gentamicin, Aminosidin and Ampicillin on the motor activity of the uterus of adult guinea-pig and dog was studied. Gentamicin induced an excitatory effect, while Aminosidin and Ampicillin induced an inhibitory action on uterine muscle, normally or stimulated by transmural stimuli. Action was similar on non-pregnant and pregnant uterus, or anestrous, estrus, and "sensitized" animals. Failure of α- and β-blocking agents, parasympatholytic, antihistaminic, and antiserotoninic agents, to prevent antibiotic action, supports the hypothesis that antibiotics act directly on the uterine muscle.

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