Action of Antibiotics on Respiratory Tract IV: Tetracyclines and 4-Epiderivatives

G. BENZI, F. BERTÉ, E. ARRIGONI, A. FERRARA, and L. SANGUINETTI

Abstract □ 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 ≥ doxycycline. The time–action relationships *in situ* indicate three distinct phases in tetracycline activity on the respiratory tract: (*a*) a *hyperventilation phase* 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 min. The intensity of action and the length of the phases are affected by: (*a*) the used tetracycline, the order of potency being rolitetracycline ≫ tetracycline > doxycycline ≥ mepicycline; (*b*) the dose employed: the larger the level, the greater the maximum effect and the longer the overall duration of action; and (*c*) the 4-epiderivative percentage of the used tetracyclines: the higher the epiderivative concentration, the greater the effect. *In situ*, the antibiotics act, at least in part, on the medullary respiratory center, the action being unaffected by cutting vagi and pretreatment with sympatholytic, parasympatholytic, antihistaminic, antiserotoninic, and ganglion-blocking agents.

Keyphrases □ Tetracyclines—action, mechanism in respiratory tract, time–action relationships □ Antibiotics—effects of tetracyclines on respiratory tract □ Respiratory tract—action of tetracyclines

Research on dog bronchial chains *in vitro* indicates that ampicillin, dicloxacillin, and chloramphenicol antagonize the histamine- or acetylcholine-induced spasm; ampicillin is about 10–20 times and dicloxacillin about 2–3 times less active than ephedrine, while chloramphenicol is about 15–25 times more active than ephedrine. *In situ* the intravenous injection of ampicillin induces an increase of the depth with a moderate decrease in the frequency of respiration, while dicloxacillin decreases the respiratory frequency with a moderate increase in ventilation. In spite of the myolytic activity *in vitro*, chloramphenicol induces *in vivo* a moderate decrease in ventilation and an increase in respiration rate (1–3).

The purpose of this study was to analyze quantitatively the action *in vitro* and *in vivo* of some tetracyclines on the dog's respiratory tract. The percentage of 4-epiderivatives in the employed products was also determined to correlate eventually the degree of these derivates with the pharmacological response. In fact, some syndromes associated with tetracycline toxicity in man were identified. Particularly, a Fanconi-like syndrome characterized by nausea, vomiting, acidosis, proteinuria, glycosuria, and aminoaciduria is associated with degraded tetracycline (4–8). This syndrome may be a result of the products of degradation, anhydrotetracycline, and epianhydrotetracycline. Experimentally, anhydro-4-epitetracycline induces in rats such marked functional changes as proteinuria, glycosuria, cellular debris in the urine sediment, and elevated UGOT activity. The morphologic concomitant of this syndrome in the rat and dog is a necrotic process of the convoluted tubuli in the renal cortex (9).

METHOD

The experiments were performed in the dog both *in vitro* and *in situ*. The following tetracyclines (with the indicated percent of epiderivatives) were used: tetracycline (3.2, 7.4, 13.2, and 39.4), doxycycline (6.8, 12.6, and 37.4), mepicycline (0.8, 7.2, 14.6, and 41.2), and rolitetracycline (7.4, 18.3, and 47.7).

The separation and determination of tetracyclines and the degradation products (4-epiderivatives, anhydro- and 4-epianhydroderivatives) were made according to the methods of Ley (10), Lodi *et al.* (11), and Gyanchandani *et al.* (12). The concentration of the degradation products was calculated as a percentage of the total tetracycline.

Experiments *In Vitro*—Bronchi of 45 mongrel dogs of either sex (weighing 7.8 to 12.5 kg.) were removed immediately after death. All tissues were carefully dissected and cut into rings which were tied together in chains with loops of thread. The mucosa was removed to allow greater freedom of movement of the muscle; the cartilage in each ring was cut so that only the smooth muscle bands were left joining each ring together (13). Four to six rings were suspended in a 50-ml. organ bath containing Tyrode's solution gassed with 95% oxygen and 5% carbon dioxide; the temperature was 36.5–37.3°. The tonus level of the preparations was continuously recorded by a strain-gauge lever, giving a magnification of ×15–20, tension of 200 mg., and writing on a kymograph drum. The preparations were left 2 hr. before any drugs were given. Before any doses of acetylcholine itself or tetracyclines were tested, two submaximal

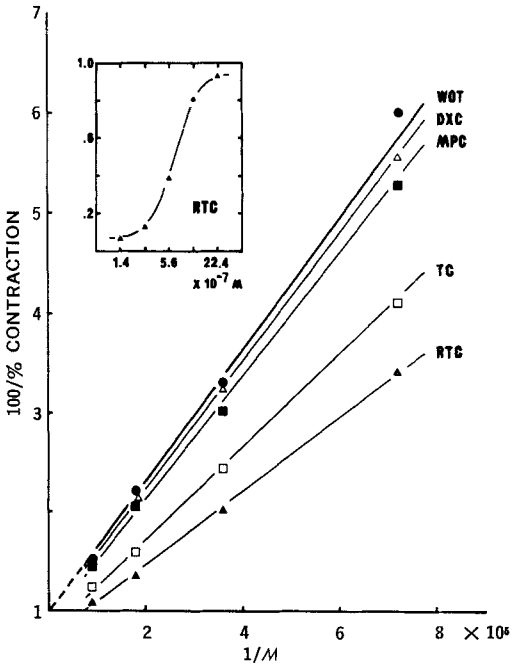

Figure 1—*Lineweaver–Burk plots calculated:* (a) *for the effect of acetylcholine chloride (2 min. of contact) on dog bronchial chain* in vitro *(WOT, ●); and* (b) *for the combinations of varying concentrations of acetylcholine chloride (1/M, in abscissa) with a constant concentration (2.8 × 10⁻⁷ M) of the tested tetracyclines introduced to the bath 2 min. before the acetylcholine doses: doxycycline (DXC, △) with 6.8% of epi-DXC; mepicycline (MPC, ■) with 7.2% of epi-MPC; tetracycline (TC, □) with 7.4% of epi-TC; and rolitetracycline (RTC, ▲) with 7.4% of epi-RTC. The insert shows the dose–response curve of rolitetracycline (for 20 min. of contact) on the dog bronchial chain.*

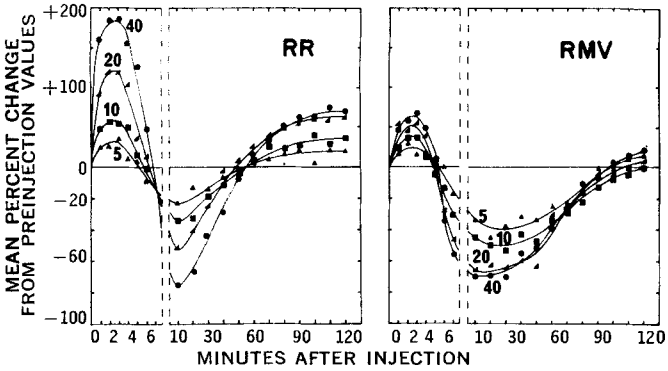

Figure 2—*Time-action curves depicting effects of injected rolitetracycline at four different dose levels (5, 10, 20, and 40 mg./kg. i.v.; 4-epiderivative = 7.4%) upon respiratory rate (RR) and respiratory minute volume (RMV). Plotted points represent average values in six dogs.*

doses of acetylcholine were administered until regular responses were obtained. The action of tetracyclines (0.7 to 22.4 $\times$ 10^{-7} *M*) was evaluated during a 20-min. period of contact.

For comparative assay, the activity of acetylcholine chloride (during 2 min. of contact) was evaluated also after the various tetracyclines (2.8 $\times$ 10^{-7} *M*) were left in contact with the preparation for 2 min.

Experiments *In Situ*—The experiments were carried out on eight beagle dogs of either sex (weighing 11.4–16.0 kg.) and on 34 mongrel dogs (weighing 8.1–12.3 kg.) preanesthetized with urethan (0.4 g./kg. i.p.). Anesthesia was induced and maintained by chloralose (80 mg./kg. i.v.); the arterial blood pressure was measured from a cannula inserted into a femoral artery; the intestinal movement and tone were recorded by a rubber balloon inserted into the jejunum.

During the succinylcholine chloride (1 mg./kg. i.v.) action, an intratracheal Warne tube was set in place; both the respiratory rate and respiratory minute volume were evaluated. All experiments were carried out in a conditioned operating room (26 $\pm$ 1°; R.H. = 55 $\pm$ 3%; pressure = 758 $\pm$ 5 mm. Hg). Immediately after completing the preinjection control measurements, the tetracyclines (5–40 mg./kg.) were administered intravenously by a polystan tube inserted into the femoral vein. The effects of the antibiotics on the respiratory tract were calculated as mean percent change from preinjection values.

To analyze the mechanism of action, tetracyclines were assayed in 18 dogs after: (*a*) cutting the vagi; (*b*) treating with the following drugs: atropine sulfate (2–3 mg./kg. s.c.), dibenamine hydrochloride (3–6 mg./kg. i.v.), D(−)-1-(4-nitrophenyl)-2-isopropylaminoethanol hydrochloride (INPEA: 4–8 mg./kg. i.v.), methysergide maleate (20–40 mcg./kg. s.c. + 10 mcg./kg. i.v.), chlorpheniramine maleate (2–4 mg./kg. s.c.), cyproheptadine hydrochloride (200–400 mcg./kg. i.v.), and hexamethonium bromide (200–400 mcg./kg. i.v.); and (*c*) giving artificial ventilation.

RESULTS AND DISCUSSION

Tetracyclines induce an increase of the motility and the tone of the dog bronchial chain *in vitro*; rolitetracycline and tetracycline act mainly on tone and motility, while doxycycline and mepicycline act mainly on motility, the tone being slightly affected or unaffected.

The order of potency in the excitatory activity on the bronchial muscle is rolitetracycline $\gg$ tetracycline $\gg$ mepicycline $\geq$ doxycycline. In fact, the Lineweaver–Burk plots in Fig. 1 show this order of ability of the mentioned tetracyclines to increase the contracting action of acetylcholine. These data are in agreement with the previous observation that tetracyclines act directly on the bile-duct smooth muscle (14).

In situ, the tetracycline effect changes with time; there are three distinct phases in tetracycline time–action curves. Following the intravenous administration of the antibiotic, a *hyperventilation phase* occurs at first for a few minutes, with time to peak effect from 2 to 3 min. After 4–8 min., a *depression of respiratory activity phase* appears. The length of this second phase is affected by such factors as the tetracycline used, dose, and epiderivative concentration; the duration of action varies from 60 to 90 min. Subsequently, a *compensatory phase* occurs with return to the control condition after 90–180 min.

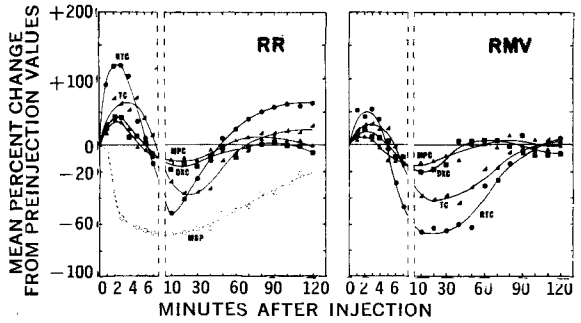

Figure 3—*Time-action curves depicting effects of rolitetracycline (RTC; 4-epiderivative = 7.4%), tetracycline (TC; 4-epiderivative = 7.4%), doxycycline (DXC; 4-epiderivative = 6.8%), and mepicycline (MPC; 4-epiderivative = 7.2%) at the same dose level (20 mg./kg. i.v.) upon respiratory rate (RR) and respiratory minute volume (RMV) and upon mean blood pressure (MBP; only for rolitetracycline). Plotted points represent average values in six dogs.*

As exemplified in Fig. 2 for rolitetracycline, the dosage influences the time–action relationships: the larger the dose, the greater the maximum effect and the longer the overall duration of action.

There is less general uniformity in the characteristics of time–action relationships depending on the tetracycline used: the position of the curve can shift along the abscissa. Nevertheless, in Fig. 3 it is possible to observe the difference in ability of various tetracyclines to induce the change in respiratory activity. The order of potency is rolitetracycline >> tetracycline > doxycycline $\geq$ mepicycline, the epiderivative concentration being quite equal in the used preparations (from 6.8 to 7.4%).

The role of epiderivatives in affecting the respiratory activity is exemplified for mepicycline in Fig. 4; for all tetracyclines, at the same dose level, the higher the epiderivative concentration, the greater is the effect. The increase in response may also result from the increase in total tetracycline, and any of the compounds studied may have caused this effect. The increased percent of the epiderivative may cause the respiratory effect in a way similar to how the increased percentage of the epiderivative causes the Fanconi syndrome.

The action of tetracyclines on the respiratory tract occurred simultaneously with a change in the arterial pressure. As indicated in Fig. 3 for rolitetracycline, a depression of the arterial blood pressure—systolic, diastolic, and mean pressure—occurs. This action was, at least in part, direct on the cardiovascular system, because it occurred also when the dogs were given artificial ventilation. On the other hand, tetracycline activity on the respiratory tract is unaffected by both cutting of the vagi and pretreatment with atropine, dibenamine, INPEA, methysergide, chlorpheniramine, cyproheptadine, and hexamethonium showing an action, at least in part, on the medullary respiratory center.

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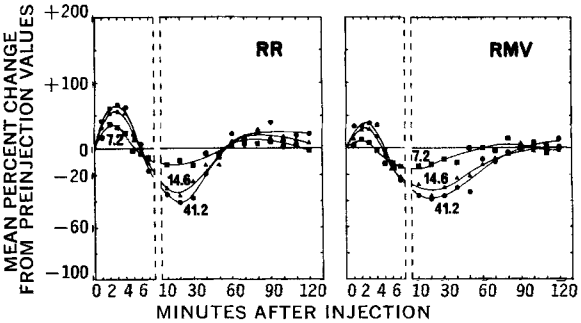

Figure 4—*Time-action curves depicting effects of mepicycline at the same dose level (20 mg./kg. i.v.) but with three different concentrations of 4-epiderivative (7.2, 14.6, and 41.2%) upon respiratory rate (RR) and respiratory minute volume (RMV). Plotted points represent average values in six dogs.*

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Potential Antidiabetics VIII: 4-Arylhydrazono-*N'*-guanylnitrate-3-methyl-2-pyrazolin-5-ones, 4-Arylazo-*N'*-guanylnitrate-3,5-dimethylpyrazoles, and 4-Arylazo-*N'*-guanylnitrate-3,5-diphenylpyrazoles

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Abstract □ A series of 4-arylhydrazono-*N'*-guanylnitrate-3-methyl-2-pyrazolin-5-ones was synthesized for evaluation as antidiabetic agents from appropriate ethyl 2,3-dioxobutyrate 2-arylhydrazones and amino guanidine nitrate. Similarly, other series of compounds, *i.e.*, 4-arylazo-*N'*-guanylnitrate-3,5-dimethylpyrazoles, and 4-arylazo-*N'*-guanylnitrate-3,5-diphenylpyrazoles were synthesized by the cyclization of 3-arylhydrazono-2,3,4-pentanetriones and 1,3-diphenyl-2-arylhydrazono-1,2,3-propanetriones with amino guanidine nitrate, respectively.

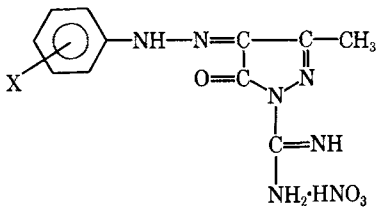
Keyphrases □ Pyrazole congeners—synthesis, screened for antidiabetic activity □ Antidiabetics, potential—pyrazole congeners synthesized, screened □ Hypoglycemic agents—pyrazole congeners synthesized, screened

Prompted by the observation (1) that 3,5-dimethylpyrazoles and isoxazoles lower the blood sugar, numerous congeners of pyrazoles were synthesized and their activity was assessed in these laboratories (2, 3). As a result of the role of *ν*-guanidobutyramide in lowering blood sugar and urea levels (4), the authors prepared several of the pyrazoles (I and II) containing two different biologically active moieties—*viz.*, guanyl and pyrazolyl.

4-Arylhydrazono-*N'*-guanylnitrate-3-methyl-2-pyrazolin-5-ones (Table I) were synthesized by the addition of a hot aqueous solution of amino guanidine nitrate to ethyl 2,3-dioxobutyrate 2-arylhydrazones under conditions similar to those used previously (5, 6).

4-Arylazo-*N'*-guanylnitrate-3,5-dimethylpyrazoles (II*a*) and 4-arylazo-*N'*-guanylnitrate-3,5-diphenylpyrazoles (II*b*) could not be obtained under these conditions, and a modified procedure was adopted. A hot aqueous solution of amino guanidine nitrate was added to an ethanolic solution of 3-phenylhydrazono-2,3,4-pentanetrione followed by 30% nitric acid until the pH of the reaction mixture became 1. The crystalline 4-phenylazo-*N'*-guanylnitrate-3,5-dimethylpyrazole started precipitating out after the reaction mixture was refluxed for 3 hr. and was allowed to stand for several hours at room temperature. By following similar conditions, other 4-arylazo-*N'*-guanylnitrate-3,5-dimethylpyrazoles and 4-arylazo-*N'*-guanylnitrate-3,5-diphenylpyrazoles were obtained. These derivatives are listed in Tables II and III.

All these compounds are highly colored and crystalline substances and are soluble in common organic solvents as well as in hot water.



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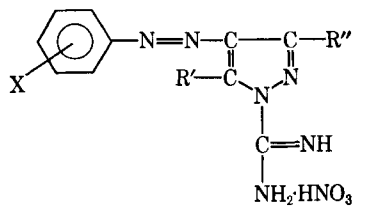

II*a*: R′ = R″ = CH_3

II*b*: R′ = R″ = C_6H_5

X = substituted phenyl