Action of Antibiotics on Respiratory Tract I: Ampicillin

G. BENZI, E. BERMUDEZ, and E. ARRIGONI

Abstract □ *In vitro* ampicillin antagonizes the barium chloride-, histamine-, and acetylcholine-induced spasm on the dog bronchial chains. Ampicillin is more effective in preventing a histamine than an acetylcholine bronchospasm, while isoprenaline and adrenaline are similarly potent against the effects of the two agonists. *In situ* the antibiotic induces at first a prompt but short increase in ventilation; subsequently, a lesser but persistent activity remains for 2 or 3 hr.

Keyphrases □ Ampicillin effect—respiratory tract □ Histamine-, barium chloride-, acetylcholine-induced bronchospasm—ampicillin effect □ Respiratory ventilation—ampicillin effect

In previous research, the action of some antibiotics *in vitro* and *in situ* upon the motility and tone of the extrahepatic biliary tract (rifamycin SV, erythromycin, tetracyclines, ampicillin, aminosidin, spiramycin, and dicloxacillin) and the ureter (ampicillin and dicloxacillin) was studied. It was particularly observed that ampicillin relaxed the above-mentioned musculatures, normal or hypertonized by barium chloride, histamine, serotonin, carbachol, or cholecystokinin (1, 2). This behavior points out the problem of the action of the antibiotics on various tissues, including the respiratory tract.

The purpose of the present paper is to report the ampicillin action on the airways *in vitro* and *in situ*. In this field, the *in vitro* pharmacological preparation consisting of a chain of tracheal or bronchial rings has been used (3, 4). Human, guinea pig, and dog preparations are equally sensitive to contracting agents, such as histamine and acetylcholine (5), and the activity of the drugs on isolated human bronchial chains agrees closely with that found by other workers using preparations from animals, including the dog (6). *In situ* the changes in airway smooth muscle tone may be measured: (*a*) by recording the dynamic pressure–volume relationship of the lungs (7), the resistance to airflow in the trachea isolated *in situ* or in the respiratory tract (8, 9); and (*b*) by the radiography of the airways (10).

The direct record of the ventilation by a rubber balloon inserted into a primary or secondary bronchus was used. For the experiments *in vitro* and *in situ*, the dog was chosen because of its size and availability.

METHOD

The experiments were performed in the dog both *in vitro* and *in situ*. Ampicillin sodium salt, of which amounts are expressed in terms of D(−)-6-(α-amino-α-phenylacetamido)-penicillanic acid, was used.

Experiments *In Vitro*—Bronchi of 32 mongrel dogs of either sex (weighing 7.4 to 10.9 kg.) were removed immediately after death. All tissues were dissected and cut in rings which were tied together in chains with loops of cotton. The mucosa was removed to allow greater freedom of movement of the muscle. Four to six rings were suspended in a 50-ml. organ bath containing Tyrode solution gassed with 95% oxygen and 5% carbon dioxide; the temperature was 36.5–37.5°. The tonus level of preparations was continuously recorded by a strain-gauge lever, giving a magnification of ×15 to 20, tension 200 mg., writing on a kimograph drum. The preparations were left 2 hr. before any drugs were given. Three submaximal doses of acetylcholine were given until regular responses were obtained, before any doses of acetylcholine itself or other agonists were tested.

The action of ampicillin (62.5–2000 mcg./ml.) was evaluated against the stimulation by barium chloride (100–400 mcg./ml.); for the construction of the dose–response curve, ampicillin activity was taken as the percent inhibition of the recorded response area by barium chloride stimulation during a 20-min. period of contact.

For comparative assay with other well-known bronchodilators, the activity of ampicillin (2048–16,384 mcg./ml.), *l*-adrenaline (0.5–4 mcg./ml.), and *dl*-isoprenaline (0.125–2.0 mcg./ml.) was evaluated against the stimulation by acetylcholine chloride (0.2–2 mcg./ml.) and histamine acid phosphate (1.0–10 mcg./ml.). Two-dose levels of agonist and antagonist were usually used; the agonists were left in contact with the preparation for 0.5–1 min. Ampicillin was introduced into the bath 10 min., and adrenaline or isoprenaline 30 sec. before the acetylcholine or histamine doses. The ED_{50}, the slope of the curve, the potency ratio, and their 95% confidence limits were calculated (11).

Experiments *In Situ*—The experiments were carried out on 28 beagle dogs of either sex (weighing 10.7–16.8 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. Through this tube a little rubber balloon was pushed into a primary or, if possible, secondary bronchus under X-ray examination.

Arterial blood pressure and intestinal and bronchial activities were recorded by a polygraph Physioscript EE12 Schwarzer.

Ampicillin (1.25–80 mg./kg.) was administered intravenously by a polystan tube inserted into the femoral vein. Two submaximal doses of *dl*-isoprenaline or *l*-adrenaline were given i.v. until regular responses were obtained before any doses of ampicillin sodium salt were tested. To analyze the mechanism of action, ampicillin was tested after: (*a*) cutting the vagi, and (*b*) treatment with the following drugs: morphine hydrochloride (4–8 mg./kg. i.v.), atropine sulfate (2–3 mg./kg. s.c.), dibenamine hydrochloride (2–5 mg./kg. i.v.), D(−)INPEA (4–8 mg./kg. i.v.), chlorpheniramine maleate (2–3 mg./kg. s.c.), cyproheptadine hydrochloride (300–600 mcg./kg. i.v.), hexamethonium bromide (300–600 mcg./kg. i.v.).

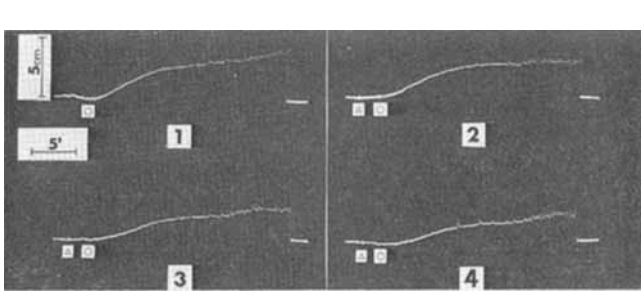

Figure 1—*Action of ampicillin on dog bronchial chain stimulated by barium chloride. The barium chloride (250 mcg./ml.) was added to the bath at the marks* (○). *The antibiotic* (△) *was present in the bath at the following concentrations: 1 = 0 mcg./ml.; 2 = 250 mcg./ml.; 3 = 500 mcg./ml.; 4 = 1000 mcg./ml. At* △ *the recording apparatus was stopped for the first 8 min. of contact with ampicillin.*

Table I—Dog Bronchial Chain *In Vitro:* ED_{50}, Slope Function of the Line (*S*), and Estimated Relative Potency (ERP), with 95% Confidence Limits, of *dl*-Isoprenaline, *l*-Adrenaline, and Ampicillin against the Stimulation by Acetylcholine Chloride (0.2–2 mcg./ml.) and Histamine Acid Phosphate (1.0–10 mcg./ml.)

Antagonist	Agonist: Acetylcholine ED_{50}[a]	Acetylcholine S[b]	Acetylcholine ERP[c]	Histamine ED_{50}[a]	Histamine S[b]	Histamine ERP[c]
Isoprenaline	0.40 (0.20–0.80)	2.30 (0.92–5.75)	28,000 (9650–81,200) D	0.49 (0.25–0.95)	2.57 (1.03–6.42)	9490 (3490–25,810) D
Adrenaline	1.30 (0.62–2.73)	2.29 (1.04–5.04)	8615 (2920–25,415) D	1.05 (0.51–2.15)	2.25 (1.00–5.06)	4430 (1575–12,450) D
Ampicillin	11,200 (5090–24,640)	2.39 (0.68–8.36)	1	4650 (2214–9765)	2.80 (0.62–12.60)	1

[a] ED_{50} = dose (mcg./ml.) of bronchodilator necessary to reduce by 50% the contraction produced by acetylcholine or histamine. [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 antagonist, and probit percent reduction of agonist activity. [c] D = ampicillin and compared drug differ significantly in potency ($p < 0.05$).

Table II—Dog Ventilation *In Situ:* Range of the Percent Changes both in Amplitude of the Recorded Tracing and in Frequency of the Respiratory Rate Induced by the i.v. Injection of Ampicillin

Ampicillin Dose Levels Injected i.v., mg./kg.	Number of Tested Dogs	Period of Observation after Injection of Ampicillin: From 0 to 20 min. Increase in Amplitude, %	From 0 to 20 min. Decrease in Frequency, %	From 20 to 60 min. Increase in Amplitude, %	From 20 to 60 min. Decrease in Frequency, %	From 1 to 2 hr. Increase in Amplitude, %	From 1 to 2 hr. Decrease in Frequency, %
1.25–5	6	24–115	8–28	10–62	8–27	0–29	0–24
10 –20	4	62–150	18–35	22–75	12–40	0–40	4–42
40 –80	4	48–174	13–38	14–94	16–43	13–48	12–37

RESULTS AND DISCUSSION

On the dog bronchial chains *in vitro*, ampicillin (62.5–2000 mcg./ml.) reduces or inhibits the stimulation by barium chloride (Fig. 1); the log dose–response curve is indicated in Fig. 2.

The comparative assay of ampicillin, isoprenaline, and adrenaline action against the stimulation by acetylcholine or histamine is summarized in Table I and shows that isoprenaline is more active than adrenaline and ampicillin in reducing the agonist-induced spasm. It is possible to observe that against acetylcholine-induced contraction, ampicillin is about 1/8600 as active as *l*-adrenaline and about 1/28,000 as active as *dl*-isoprenaline. According to Mc Dougal and West (5), on dog tracheal rings aminophilline possesses about 1/10,000 the activity of adrenaline and about 1/40,000 the activity of isoprenaline. On the other hand, ampicillin, as is aminophilline (5), is more effective in preventing a histamine than an acetylcholine bronchospasm, while adrenaline and isoprenaline are similarly potent against the effects of the two agonists.

Obviously the *in vitro* technique employed provides information concerning potency ratio or mechanisms operant in the airway wall; nevertheless it is unphysiological, the studies being limited to denervated portions of the airways. However, it is impossible to correlate directly the present results with clinical conditions. In fact it is well known that many bronchoconstrictive stimuli act, at least in part, *via* reflex mechanisms, and these effects are absent in the *in vitro* preparation. Nevertheless, the results *in vitro* related to isoprenaline and adrenaline agree with the findings of previous workers (12) using *in vivo* the test of the antagonism of the effects of a histamine aerosol in guinea pigs.

The observations *in situ* indicate that, after intravenous injection of 1.25–80 mg./kg., ampicillin induces a biphasic action. At first the antibiotic induces a prompt, high, but short (5–20 min.) increase in ventilation, and subsequently a lesser but persistent activity remains for 2 or 3 hr., as summarized in Table II and exemplified in Fig. 3. No change in systemic blood pressure occurs during the ampicillin action on the airways.

The increase in ventilation induced by ampicillin during its first phase of action is similar to those caused by the i.v. injection of isoprenaline (0.125–2 mcg./kg.) and adrenaline (0.5–5 mcg./kg.). Nevertheless, related to the very different biotransformation, the total time of activity of the sympathomimetic amines is much shorter (2–8 min.) than that of ampicillin, and furthermore the well-known changes in systemic blood pressure occur.

The *in situ* preparation utilized by the authors does not provide any information on drug effects on peripheral airways which are vitally concerned with the gas-exchange process. The pressures recorded by the bronchial balloon must have been changes in the depth of respiration, and it is completely possible that the results

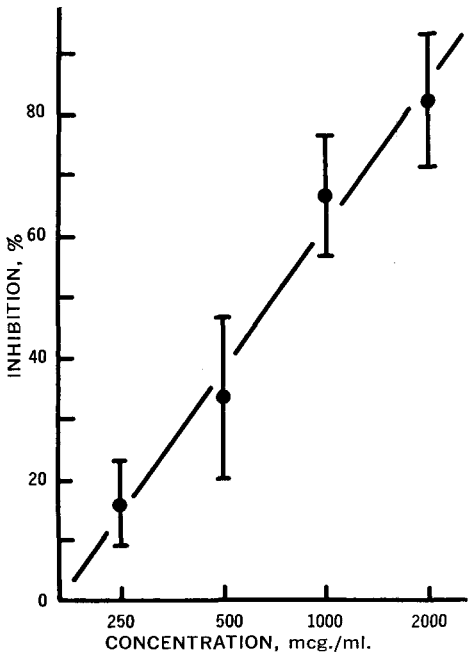

Figure 2—*Log dose–response curve of ampicillin action against the stimulation by barium chloride. The ordinate shows the inhibition percent of the contracting action by barium. The concentrations of the antibiotic (mcg./ml.) are plotted on the abscissa in logarithmic scale. The vertical lines indicate standard errors of the means. Eight preparations at each dose level.*

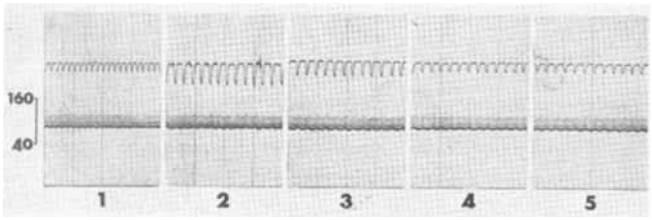

Figure 3—*Action of ampicillin i.v. on the ventilation of a dog* in situ. *From top to bottom: tracing recorded by a balloon inserted into the bronchus; blood pressure (mm. Hg); 1 = control condition; 2 = 2 min. after injection i.v. of 10 mg./kg. of ampicillin; 3 = 30 min. later; 4 = 1 hr. later; 5 = 2 hr. later.*

are only in part associated with changes in bronchial tone. In fact, it should be noted that: (*a*) the baseline of bronchial balloon pressure did not change appreciably in response to ampicillin, indicating that the resting tone of bronchial muscle was slightly or not affected by the drug; (*b*) the ampicillin action was antagonized by the high respiratory depression by morphine; and (*c*) the antibiotic action was unaffected after treatment with atropine, dibenamine, INPEA, chlorpheniramine, cyproheptadine, and hexamethonium.

In conclusion, only *in vitro* is it possible to postulate the ability of ampicillin to relax directly the bronchial musculature. *In vivo* the pressure fluctuations noted with each breath were probably not caused by rapid breath-to-breath changes in bronchial tone. It is more likely that ampicillin increased the activity of the respiratory center, causing an increase in ventilation and a subsequent increase in anatomic deadspace.

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Determination of Epitetracycline and Chlortetracycline in Tetracycline by Quantitative Thin-Layer Chromatography

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Abstract □ Small amounts of epitetracycline and chlortetracycline in tetracycline were determined by quantitative thin-layer chromatography on kieselguhr layers, impregnated with a citrate–phosphate solution, pH 5.5, containing 10% glycerin. The method involves chromatography under sharply defined conditions (relative humidity, temperature, and rapidity of spotting) in order to obtain a good separation of the different zones and to prevent rapid epimerization during development. After elution with 0.1 *N* HCl, epitetracycline was measured at 356 mμ, while for chlortetracycline the fluorimetric method of Chicarelli was used. The possible identities of three other impurities—with R_f values between epitetracycline and tetracycline—are discussed; their percentages were calculated as epitetracycline.

Keyphrases □ Epitetracycline—analysis, separation in tetracycline □ Chlortetracycline—analysis, separation in tetracycline □ TLC—analysis □ Spectrophotometry—analysis

In 1963, Remmers *et al.* (1) described a spectrophotometric determination of 4-epitetracycline (epi-TC) in tetracycline (TC), based on the absorbance-ratio difference at 254 and 267 mμ. However, this method is not suitable for measuring small amounts of epi-TC in commercial samples; accurate measurements at those wavelengths are disadvantageously affected by the presence of anhydrotetracyclines (ATC, epi-ATC). The paper chromatographic method, reported by Addison and Clark (2), gives better information about the percentage of epi-TC.

When small amounts of chlortetracycline (CTC) are to be determined, the spectrophotometric procedure, given by Woolford *et al.* (3), is not preferable. It is also not possible to apply the fluorimetric procedure [Chicarelli (4)] because the fluorescence of the CTC is quenched by the TC solution.

In 1964, Sonanini and Anker (5) described the identification of three tetracyclines on kieselguhr layers, impregnated with a solution (pH 3.7) containing 5% glycerin. In 1968, Ascione *et al.* (6) reported the separation of some tetracyclines on the same support, impregnated with a EDTA–PEG 400–glycerin solution, pH 7.0.

The present authors used the idea of Sonanini and Anker as a starting point for a quantitative determination of epi-TC and CTC present in commercial samples of TC.