PHARMACOLOGICAL STUDY OF A NEW ANTIBIOTIC OF BACILLARY ORIGIN

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The purified extract of a new bacterial mutant, which exhibits antibiotic activity against paramecia and amoebae, was tested on various smooth muscle preparations. Spontaneous activity as well as the response to a variety of smooth muscle stimulants was diminished or completely suppressed. Intravenous injection of the extract lowered the blood pressure of the anaesthetized cat or dog. These properties may explain the beneficial effect of the extract on the intestinal symptoms of human amoebiasis, before eradication of the parasites.

In 1946, Reitler and Boxer isolated a new bacterial strain from the mesenteric lymph glands of a patient who succumbed to ileus with multiple intussusceptions of the jejunum. This strain grew in the form of short, Gram-negative rods, which produced spores abundantly. It also showed interesting antibiotic properties and exhibited a marked tendency to mutate under a variety of experimental conditions. Systematic selection led to mutants, which possessed not only antibacterial activity but produced also antiprotozoal substances in increasing quantities. One of these mutants, labelled RB-103, distinguished itself by its filamentous growth, its weak tendency to sporulation and its regular production of material, active against paramecia and even to a higher degree against amoebae (Reitler and Berner, 1960).

Purified extracts from the sub-strain RB-103 have been tried on several hundred patients suffering from acute or chronic infection with *Entamoeba histolytica*. Marked improvement of the intestinal symptoms was usually noted after a few days of treatment, but examination of the stools at this stage still revealed the presence of numerous amoebae and cysts. Analysis of the faeces became negative only after 2 to 3 weeks of continuous administration of the antibiotic. Such observations led to the idea that, in addition to the antibiotic substances, the bacterial extract may contain principles that act on the smooth muscle of the intestine and thus could be responsible for the pronounced change of the clinical picture long before elimination of the parasites (Reitler, 1950).

The experiments described in this paper were designed to test this hypothesis and to investigate in general the pharmacodynamic properties of the antibiotic of the new strain.

METHODS

Antibiotic Material.—A sterile extract of the mutant RB-103, representing the last stage in the purification process, immediately before adsorption on to a solid carrier, was put at our disposal by Messrs. Hillel Remedies Inc., Haifa. The antibiotic activity of the preparation was tested against *Paramecium caudatum*, which is first immobilized and, during 24 hr., undergoes lysis. The extract used caused complete lysis at a dilution of 1 in 200. For the sake of brevity, we shall use in this paper the commercial name "Colisan" for the antibiotic preparation.

Isolated Organs.—Smooth muscle organs were suspended in an organ bath at 38°, containing aerated Locke solution. When the response to graded doses of a stimulant was irregular (for example, with the ileum of the guinea-pig), it was advantageous to store the organ in the refrigerator overnight. In the experiments described, figures about reagents refer to the final concentration of the substance in Locke solution.

Whole Animals.—Cats and dogs were anaesthetized with intravenous pentobarbitone, after induction with ether. Blood pressure was recorded from the left carotid artery, using an Hg manometer, and respiration by a membrane manometer, connected to the side arm of the tracheal cannula. Contractions of the left gastrocnemius were produced by stimulation of the sciatic nerve, placed on shielded silver electrodes, and registered by attaching the muscle to an isometric lever.

FIG. 1.—Antagonism of acetylcholine and colisan on the isolated guinea-pig ileum. A, Acetylcholine, 0.004 $\mu\text{g./ml.}$ B, Colisan, 1 in 100 final dilution. C, Without washing, addition of acetylcholine, 0.004 $\mu\text{g./ml.}$ D, Without washing, addition of acetylcholine, 0.16 $\mu\text{g./ml.}$ Between D and E, repeated washings. E, Acetylcholine 0.004 $\mu\text{g./ml.}$ Time marker, 1 min.

RESULTS

Effect of Colisan on the Guinea-pig Ileum.—An intestinal loop, incubated with colisan, showed a progressively diminishing response to acetylcholine. After 5 to 7 min. complete inhibition was obtained (Fig. 1). This effect was reversible; after repeated washing, the loop returned to its original sensitivity. The effect of colisan is competitive with the stimulant drug, because concentrations of acetylcholine, 10 to 50 times higher than the one used before inhibition, provoked a response even after 10 min. incubation with the bacterial extract.

The inhibitory effect can also be demonstrated in the reverse manner. Addition of colisan at the height of the contraction, due to stimulation by acetylcholine, led to immediate relaxation of the ileum (Fig. 2). This reverse reaction is distinguished from the effect of previous incubation not only by the time factor but also by the lower dose of colisan needed for complete relaxation (about 1/3 to 1/5 of the dose required in the incubation method).

The reverse method, because of its immediate effect, lends itself to a quantitative estimation of the activity of colisan. Increasing doses of the drug produce an increasing spasmolytic action (Fig. 3). By plotting log colisan concentration

against the percentage decrease of the amplitude of the contraction induced by acetylcholine, a straight line is obtained (Fig. 4).

Effect of Colisan on the Guinea-pig Colon.—Analogous observations were made on the isolated colon. The spontaneous peristaltic movements of the organ, as well as the contractions produced by various stimulants, were abolished by colisan, which clearly exerts a threefold action (Fig. 5): (a) It lowers the tonus of the smooth muscle; (b) it diminishes the amplitude of the contractions; and (c) it reduces the frequency of the peristaltic movements.

Non-specificity of the Antagonism Between Colisan and Stimulants of Intestinal Smooth Muscle.—Colisan exerts its effect against any one of the following stimulants: acetylcholine, histamine, 5-hydroxytryptamine, nicotine, γ -amino-butyric acid and barium chloride. When equiactive doses of stimulants were compared, approximately the same concentration of colisan was required to counteract their effect. The broad antispasmodic spectrum demonstrates the non-specificity of colisan, compared with the action of other known antagonists of smooth muscle stimulants. Thus, a dose of atropine sufficient to abolish the response to acetylcholine will

FIG. 2.—Effect of colisan, added at the height of a contraction of the guinea-pig ileum caused by acetylcholine. A, Acetylcholine, 0.02 $\mu\text{g./ml.}$ B, Without washing, addition of colisan, 1 in 50 (final dilution). Between B and C, repeated washings. C, Acetylcholine, 0.02 $\mu\text{g./ml.}$

FIG. 3.—Graded effect of colisan on the contractions of the guinea-pig ileum, induced by 0.02 $\mu\text{g./ml.}$ of acetylcholine. 1, Control. 2, Colisan 1 in 200. 3, 1 in 165. 4, 1 in 145. 5, 1 in 125. 6, 1 in 110. Note that 5 already produced maximal relaxation, while 6 produced this effect within a shorter time.

suppress only partially the response to histamine, 5-hydroxytryptamine, nicotine, or barium chloride. A given dose of the antihistamine diphenhydramine, which eliminates completely the contractions of the ileum induced by histamine or nicotine, is only partially effective against acetylcholine or 5-hydroxytryptamine. Florey's factor I shows specific antagonistic activity against acetylcholine and nicotine, but does not influence the response to 5-hydroxytryptamine (Florey and McLennan, 1959). Finally, botulinum toxin abolishes the contraction caused by nicotine, but does not interfere with the effect of acetylcholine or histamine (Ambache and Lessin, 1955).

Effect of Colisan on Other Smooth Muscle Organs.—The above experiments show that the relaxing effect of colisan is independent of the

FIG. 4.—Percentage relaxation of guinea-pig ileum as function of log drug concentration. Taken from the data of Fig. 3. Ordinate, $\frac{\text{amplitude of descending limb of trace} \times 100}{\text{maximal amplitude of ascending limb}}$

specific transmitter mechanism which operates in a given organ. This view is further supported by the observation that all smooth muscle organs tested showed essentially the same response to colisan as does the intestine. A survey of the organs examined and the agents used as most effective stimulants in each case is given in Table I, which also shows the species independence of the drug effect. For example, the non-pregnant uterus of the guinea-pig, which responds best to acetylcholine and histamine, and the uterus of the rat in the third week of pregnancy, which contracts after acetylcholine, 5-hydroxytryptamine (Fig. 6), and oxytocin, were both completely inhibited by colisan.

FIG. 5.—Antagonism of acetylcholine and colisan on the guinea-pig colon. The movements, left of A, represent the spontaneous peristalsis of the colon. A, Acetylcholine, 0.01 $\mu\text{g./ml.}$ B, Without washing, addition of colisan 1 in 25 final dilution. Between B and C, repeated washings. Left of C, recovery of spontaneous peristalsis. C, Acetylcholine, 0.01 $\mu\text{g./ml.}$

TABLE I
SMOOTH MUSCLE ORGANS TESTED FOR THE
ANTAGONISTIC EFFECT OF COLISAN

The tests with γ -aminobutyric acid were carried out according to Hobbiger (1958).

Species	Organ	Stimulants Used
Guinea-pig	Ileum	Acetylcholine, histamine, 5 - hydroxytryptamine, nicotine, γ -aminobutyric acid, barium chloride
„	Colon	Acetylcholine, histamine, 5 - hydroxytryptamine, nicotine, barium chloride
„	Uterus (non-pregnant)	Acetylcholine, histamine, barium chloride
„	Ureter	Histamine, nicotine, barium chloride
„	Bladder	Histamine, barium chloride
Rat	Uterus (third week of pregnancy)	Acetylcholine, 5-hydroxytryptamine, oxytocin, barium chloride
„	Stomach fundus	5-Hydroxytryptamine

FIG. 6.—Horn of uterus of rat in third week of pregnancy. 1, 0.05 $\mu\text{g./ml.}$ 5-hydroxytryptamine. 2, Colisan 1 in 100. After 7 min. incubation, addition of 0.05 $\mu\text{g./ml.}$ 5-hydroxytryptamine at 3.

FIG. 7.—Effect of intravenous injections of colisan on the blood pressure of the dog under nembutal anaesthesia. 1, 0.5 ml. colisan. 2, 1.0 ml. colisan. 3, 2.0 ml. colisan.

Effect of Colisan on Striated Muscle.—Intravenous or intra-arterial injection of colisan into the cat had no effect on the amplitude of the contractions of the gastrocnemius, stimulated indirectly. Similar results were obtained with the phrenic-diaphragm preparation of Bülbring (1946).

Effect of Colisan on the Blood Pressure.—The response of the smooth muscle of the arterial wall was tested by measuring the blood pressure in the intact dog or cat. As shown in Fig. 7, after intravenous injection of colisan, the blood pressure fell immediately and returned within 1 to 2 min. to its original level. However, thereafter the blood pressure fell progressively to very low levels. Infusion of adrenaline raised the pressure again and restored the hypotensive response of the animal to colisan.

DISCUSSION

The extract from the bacterial mutant RB-103 contains a smooth muscle relaxant with a spectrum of activity much broader than that of other known antagonists to chemical stimulation, whether of animal, plant, or bacterial origin. The inhibitory efficiency of colisan against a whole array of smooth muscle stimulants, independent of their specific point of attack along the neuronal system or at the effector cell itself, supports the view that the drug acts directly on the muscle cell. The important fact should be stressed that, after

incubation with colisan, any of the stimulating drugs tested can provoke contraction, if doses 10 to 50 times higher than the original ones are applied. Apparently, the antagonistic effect takes place at the contractile substance itself, a view supported by the observation that equal concentrations of colisan were required to neutralize the effect of equiactive doses of different stimulants.

The peculiar character of colisan is also revealed by comparison with other antagonists against smooth muscle stimulants. Florey (1954) isolated from brain extracts Factor I, which inhibits movements of the gut caused by acetylcholine or nicotine, but has no effect on the contraction provoked by 5-hydroxytryptamine (Florey and McLennan, 1959). In addition, these authors noted that about 25% of their biological preparations were insensitive to the brain extract. These properties distinguish Factor I from colisan, in view of the broad antagonistic spectrum of the latter (see Table I) as does the fact that its solutions, if active against one specific organ, are effective against all organs of the same or different species as far as has been tested.

The point of attack of colisan is also clearly demarcated from that of the botulinum toxins (Ambache, 1951). The latter abolishes contractions caused by nicotine, but does not impair the effect of acetylcholine, thus suggesting the neuronal parts of the preparation, that is, the synapse of the outer ganglion cells, the post-ganglionic neurons in the wall of the gut, or the nerve terminals at the muscle surface as locus of action. Furthermore, after about 10 min. incubation, the toxin cannot be washed out any more, whereas the effect of colisan is completely reversible by washing.

The situation is different with respect to staphylococcal β -toxin, which suppresses completely the spontaneous peristalsis of the isolated rabbit intestine as well as its response to acetylcholine (Anderson, James, and Marks, 1954; Kelsey and Hobbs, 1954). However, a comprehensive study of the localization of this antagonistic action has not been carried out. Therefore, it cannot be decided at present whether the toxin belongs to the same group as the active principle present in the extract of the mutant RB-103.

The present experiments support the hypothesis that the beneficial effect of colisan on human amoebiasis is due, at least in part, to the presence of a smooth muscle relaxant, which reduces the motility of the gut and thus alleviates the intestinal symptoms some time before the antiprotozoal effect becomes apparent. The pharmacodynamic observations with colisan naturally raise the question whether the antispasmodic principle of the bacterial extract is a separate entity or is identical with the antibiotic present in colisan. Experiments pertinent to this question will be reported in due course.

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