The Pharmacologic Action of Thevetoidin, a Cardioactive Substance Obtained from a Mexican Species of Thevetia

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Thevetoidin is a digitalis-like substance isolated from a Mexican species of thevetia. Study of its action on the isolated frog heart, on the anesthetized dog, and on the failing heart of the heart-lung preparation reveals therapeutic and toxic effects of unmistakable digitalis-like character which are quickly established and wear off rapidly. The dose for the treatment of cardiac disorders was deduced from experiments on the failing heart of the heart-lung preparation on the basis of a comparison with ouabain.

Four cardioactive substances have been obtained from Thevetia neriifolia: (1) thevetin, described by DeVry in 1863, and subjected to chemical and pharmacologic study by Chen and his collaborators; (2) neriifolin, isolated by Frerejacque and by Helfenberger and Reichstein; (3) acetylnerifolin, isolated by Frerejacque and Durgeat; and (4) thevetobioside, prepared by enzymatic hydrolysis of thevetin with strophanthobiase. Chen and Chen have also isolated from Thevetia neriifolia two substances, kokilphin and ahouain, lacking in cardiac activity.

Several species of the genus thevetia exist in Mexico, but there has been no modern study of their botanical classification and distribution, and chemical and pharmacologic studies have been scarce. Guerra has recently made a historical and botanical study of the so-called Thevetia yecotli from which he has obtained by dialysis a mixture of glycosides. He also claims to have obtained thevetin. Chen and Chen had already obtained in 1938 thevetin and kokilphin from a sample of Mexican seeds which they also classified under the name of Thevetia yecotli.

The authors, working with Thevetia gauumeri identified by M. Martinez, and using the extraction procedure described below, have obtained a moderately hygroscopic white crystalline substance for which they proposed the name of thevetoidin. The pharmacologic study of thevetoidin on the heart is described in the present paper. A preliminary clinical study of thevetoidin carried out by Chavez, Mendez and Chait demonstrated its possible use in certain cardiac disorders.

Methods

The chemical extraction of thevetoidin is accomplished in the following stages: removal of fat from the seeds with petroleum ether; extraction with cold alcohol and later with hot alcohol; evaporation of the alcohol in vacuo; repeated washing of the residue with ether until the ether contains no more colloidal substance; extraction of the residue with hot water (50 to 60°C) in vacuo; treatment with lead acetate at a temperature of 50 to 60°C in vacuo; filtration and precipitation of the lead with hydrogen sulfide at a temperature of 50 to 60°C; neutralization of the free acetic acid with sodium carbonate at a pH of 7.2; filtration and evaporation in vacuo; extraction with alcohol and purification with activated charcoal; evaporation and extraction with absolute alcohol; further purification with charcoal until the solution is completely colorless; evaporation and extraction with absolute methyl alcohol; precipitation of the active substance with anhydrous ether; centrifugation and desiccation in vacuo.

For the experiments on the isolated heart of the frog and for the determination of the half lethal dose, frogs of the variety Moctezuma were used. The heart was isolated according to the technic of Straub-Fühner. The physiologic solution used had the following composition: sodium chloride 0.65 per...
cent; potassium chloride 0.014 per cent; Calcium chloride (anhydrous) 0.011 per cent; sodium bicarbonate 0.03 per cent.

For the determination of the half lethal dose and the dose mortality curve, groups of six frogs were injected at each dose level. The injections were made into the ventral lymph sac through the muscles of the thigh. Results were analyzed according to the method of Behrens. The experiments were conducted at an environmental temperature which varied between 23 and 25°C.

The experiments in the heart-lung preparation were conducted on dogs of 8 to 13 Kg. The technic was that described by Krayer and Méndez, utilizing the Stolnikov stromuhr in place of the Weese stromuhr used by those authors. In most of the experiments cardiac insufficiency was provoked by pentobarbital (80 to 180 mg.) in divided doses of 50 mg. or less. Competence tests were used according to the technic of Krayer. A chloroform manometer was used to record pressure in the left auricle. In two experiments in which coronary blood flow was measured, a Morawitz cannula was introduced into the coronary sinus. In experiments with the innervated heart-lung preparation, the venous cannula was introduced into the inferior vena cava and the arterial cannula into the descending aorta, maintaining intact the circulation of the head. For experiments in which electrocardiographic changes were studied in the intact animal, dogs of 6 to 10 Kg. anesthetized with pentobarbital (20 mg. per Kg.) and chloralose (70 to 80 mg. per Kg.) were used. The three standard leads were used. Records were taken with a model III Grass electroencephalograph.

RESULTS

I. Isolated Frog Heart

The systolic effect of thevetoin is analogous to that of other digitalis-like substances. It produces first a positive inotropic effect, followed by a diminution of ventricular relaxation until termination in ventricular arrest with continuation for some time of auricular activity. The heart rate diminishes; frequently there is observed incomplete A-V block and at times A-V dissociation.

The essential characteristic of the action of thevetoin on the isolated heart is its reversibility. In figure 1 it may be seen that thevetoin in a concentration of 1 × 10⁻³ produced systolic arrest in six minutes. About an hour after washing, the amplitude of the contractions and the cardiac frequency had returned to normal. In other experiments complete restoration was obtained half an hour after washing with Ringer Solution. The second administration of thevetoin in the same concentration of 1 × 10⁻³ produced systolic arrest in about the same time as the first. The reversion to normal was also complete after washing. The reversibility of the action of thevetoin is more rapid as the concentration is reduced. Thus with a concentration of 1 × 10⁻⁴ complete reversion was obtained in some experiments within a few minutes.

We intended to obtain a curve plotting concentration against the time necessary for systolic standstill similar to the curves which have been obtained with digitoxin, ouabain and other substances, but we were unable to obtain a sufficient number of points, since the limiting concentration with which we were able to produce systolic arrest of the ventricles was 5 × 10⁻⁵. This concentration caused an intense sys-
tolic effect but in most of the experiments did not produce clear systolic arrest. This suggests a slight fixation of thevetoidin in the frog heart in comparison with most of the cardiac glycosides studied until now.

II. Half Lethal Dose in the Frog; Cat Unit

The half lethal dose in the frog was determined in comparison with ouabain (Arnaud) under the same experimental conditions. Eight doses of each substance were injected. The half lethal dose of the sample of ouabain used was 0.0003 mg. per Gm. weight; that of thevetoidin was 0.066 mg. per Gm. These results show that

Toxicity of thevetoidin in the frog by injection in the lymph sac is approximately one two-hundredth that of ouabain. This difference does not agree with the results obtained by intravenous injection in the cat nor with those obtained in the heart-lung preparation.

The cat unit was determined by the method of Hatcher, following the technic of Storm van Leeuwen, and by the method of the U. S. P. XIII. With the first method, the cat unit was 2.3 mg.; by the second, 2.6 mg.

III. Action of Thevetoidin in the Heart-Lung Preparation

(a) Failing Heart. In the opinion of the authors of this paper, there is no better experimental method for demonstrating a digitalis-like action of a substance than its study in the failing heart of the heart-lung preparation. This point of view has brought one of the authors\textsuperscript{15} to consider as the most decisive proof of digitalis-like action, the reversal of the cardiac failure in the Starling heart-lung preparation, with a sustained therapeutic action and the ultimate development of characteristic toxic effects. This is not to deny the merits of studies of toxicity in the cat nor of the precise electrocardiographic disturbances which may be recorded in the dog or cat under anesthesia, but there can be no doubt that reversal of experi-

Fig. 2. Action of thevetoidin upon experimental heart failure in the heart-lung preparation of the dog. Weight of the heart-lung dog, 9.2 Kg. Weight of the heart 72 Gm. Total amount of blood in the system, approximately 950 cc. Amount of blood in the reservoir when thevetoidin was injected 400 cc. Arterial resistance 82 mm. Hg. Part A, nonfailing heart; part B, failing heart. Between A and B 120 mg. of pentobarbital were injected. Tracings from top to bottom: arterial blood pressure (scale on top at left in mm. Hg.); right auricular pressure (scale on left in mm. HgO); time in half-minute intervals. The horizontal rows of figures indicate from top to bottom: systemic output in cc. per minute; heart rate per minute; temperature in degrees centigrade; raising and lowering of the reservoir in mm. HgO. At arrow 0.8 mg, of thevetoidin was injected.

mental heart failure constitutes a complementary proof and an indication of therapeutic activity not possessed by any of the other methods of study of substances with digitalis-like action.

Thevetoidin reverses completely experimental heart failure in the heart-lung preparation when administered in a minimal dose of 0.8 mg. Figure 2 shows clearly this effect. Part A of the figure shows the response of the sufficient heart to changes of venous return caused by elevation of the venous reservoir. Comparison of the response of the sufficient heart with that of the failing heart and with that of the heart restored by a cardiac glycoside constitutes the so-called competence test of Krayer.\textsuperscript{13} The
sufficient heart (part A) responds to the increase of venous return with a proportional augmentation of ventricular output and with a very slight elevation of right auricular pressure. Elevation of the reservoir in three steps of 50 mm. each increased the output of the left ventricle from 612 cc. per minute to 800, 930 and 1100 cc. per minute. Between parts A and B of this tracing the heart was made insufficient with pentobarbital and the left ventricular output diminished to 430 cc. per minute with a corresponding elevation of auricular pressure. The administration of 0.8 mg. of thevetoidin was followed by a rapid decrease of the auricular pressure and a considerable increase of cardiac output. Repetition of the competence test (right side of part B) produced results similar to those which had been obtained in the normal heart. Part C shows the reversibility of the effect of a dose of 0.8 mg. of thevetoidin in the heart-lung preparation. Elevation of venous pressure and decrease of cardiac output, indicating the return to the insufficient state, were obvious an hour after the administration of thevetoidin. With these small doses which produce complete reversal of failure, the effect disappears almost completely between 50 and 70 minutes.

In other experiments the elevated pulmonary arterial pressure resulting from left ventricular failure was also observed to decrease after the injection of thevetoidin.

(b) Toxic Action on the Sufficient Heart. The heart of the heart-lung preparation at the beginning of an experiment is generally in a mild state of insufficiency. If the heart were completely normal, the administration of a substance with a digitalis-like action would not produce any effect on the ventricular output or on auricular pressure. Figure 3 shows the effect of thevetoidin on a heart which in the laboratory might be considered normal, but which probably, under the influence of the anesthesia, the operation and the time lapse before the beginning of the experiment, had developed a mild degree of insufficiency. To this may be ascribed in part the effect of the first injection of 2 mg. of thevetoidin, which before causing toxic effects, caused a slight increase of cardiac output accompanied by a slight decrease of venous pressure. This moderate therapeutic effect was followed by toxic effects characterized by wide and rapid fluctuations of venous pressure due to the production of A-V dissociation. The cardiac output, which, under the therapeutic effect of the drug, had increased from 600 to 625 cc. per minute, decreased to 545 during this first phase of the toxic action. The mean venous pressure was elevated also as a result of the dissociation. The auricu-
lar rate diminished, probably as a result of an action on the sinoauricular node. The second injection of 2 mg. of thevetoidin augmented the toxic effects, producing a more marked increase of venous pressure and of ventricular automatism. Auricular automatism ceased under the effect of this second dose. The third injection of 2 mg. led to the production of ventricular fibrillation with a sudden fall of arterial pressure and maximal elevation of auricular pressure.

The toxic effects described are similar to those which are produced by all of the cardiac glycosides that have been studied in the heart-lung preparation.

IV. Action on the Electrocardiogram of the Anesthetized Dog

Figure 4 shows the rapid and reversible effect of thevetoidin on the electrocardiogram of the anesthetized animal. The dose injected intravenously in this experiment was 0.6 mg. per Kg. The electrocardiographic changes were recorded continuously with a direct-writing instrument (see Methods). At 85 seconds after the injection there occurred a considerable bradycardia with lengthening of the P-R interval and occasional sinoauricular block. Two minutes after the injection, the first ventricular premature contraction was recorded, with augmentation of the bradycardia and great depression of A-V conduction. At six minutes after the injection there appeared periods of A-V dissociation alternating with periods of sinus rhythm and at about seven minutes the A-V dissociation was complete. This continued until 18 minutes. Then the first sign of regression of the toxic effects was observed; periods of sinus rhythm began to be intercalated with periods of dissociation. In the periods of sinus rhythm,
the experiments the disappearance of the electrocardiographic changes was complete in 45 minutes after the injection. In one experiment in which 1 mg. per Kg. was injected the toxic effects were more intense and developed more rapidly than those indicated in the experiments of figure 4. The first extrasystoles appeared 35 seconds following the injection. The toxic effects disappeared less rapidly than with the dose of 0.6 mg. per Kg. At two hours and twenty minutes the P-R interval was still considerably longer than normal. The complete disappear-

ance of the electrocardiographic changes did not occur until three hours after the injection.

V. The Vagal Effect of Thevetoidin

The rapid development of bradycardia, the increase of the P-R interval and the sinoauricular block occasionally obtained following the injection of thevetoidin in anesthetized dogs suggested a strong vagal component in the effect of this substance. This effect was studied in three experiments with the innervated heart-lung preparation.

In the first experiment 5 mg. of thevetoidin were injected in a dog of 7 Kg., anesthetized with pentobarbital; 20 seconds later an intense bradycardia with elevation of the venous pressure occurred. Section of the vagus nerves was immediately followed by restoration of the venous pressure and of the heart rate to the values previous to the injection. In the other two experiments, in dogs anesthetized with pentobarbital-chloralose (see Methods), the result illustrated in figure 5 was observed. In these experiments 9 mg. of thevetoidin were injected in a dog of 12 Kg. The effect on the rate and the venous pressure developed gradually and when it appeared to be maximal (at two minutes and forty seconds) section of the vagus nerves was followed by a fall of the venous pressure and restoration of the heart rate to its previous level; however, the P-R interval did

not return completely to normal, indicating a direct effect on the conduction tissue. Seventy seconds after section of the vagus nerves, A-V dissociation appeared which was due to the digitalis-like action.

The vagal effect produced in these experiments was so intense that it surpassed the effect which thevetoidin might have had on contractility in the first two or three minutes of its action, since the reduction of heart rate was accompanied by an augmentation of the auricular pressure and reduction of the cardiac output. (See fig. 5.)

VI. Other Effects

(a) Vomiting and Diarrhea. In two experiments thevetoidin was injected intravenously in two dogs without anesthesia. The dose was 0.6 mg. per Kg. Both dogs vomited between
one and one-half and two minutes after the injection. One of them developed diarrhea about eight minutes following the injection. We have not studied the emetic action of thevetoidin in pigeons. We can, however, affirm that thevetoidin has an emetic action more marked than that of ouabain, since in clinical experiments\textsuperscript{10} the therapeutic dose of 0.02 mg. per Kg. produced vomiting in most of the patients.

(b) Arterial Pressure. Thevetoidin has an action on the arterial pressure similar to that of the cardiac glycosides. A dose of 0.6 to 1.0 mg. per Kg. produces an immediate elevation of arterial pressure which is sustained for 10 to 12 minutes before descending slowly to the original level. The pressure record, however, showed irregularities due to periods of A-V dissociation. Coincident with the elevation of pressure there occurred a decrease of the respiratory rate. An effect upon the arterial pressure was also recorded in the experiments used for determination of the cat unit by the method of Hatcher. The changes observed are similar to those which are seen with the other digitalis-like substances.

c) Coronary Blood Flow. In two experiments, the coronary flow was measured in the heart-lung preparation. Neither the dose of thevetoidin necessary to reverse cardiac insufficiency nor that necessary to produce cardiac irregularities caused any significant change in the flow of blood collected from the coronary sinus. We have not measured the coronary flow in anesthetized animals with the thorax opened to determine the influence which the vagal effect of the substance may exert on the coronary flow in the innervated heart.

Discussion

We have worked with four batches of thevetoidin and the characteristics of the substance have not always been the same. In the four lots we have encountered differences, although small, in the melting point and in the activity of the compound. This taken together with the clinical study,\textsuperscript{10} which revealed two distinct actions, an early transient one and a cumulative action comparable to that of strophanthin, made us think that thevetoidin is composed of more than one substance. Further chemical studies, still in progress, have revealed the presence of acetylthevetin and, in addition, a small amount of a quaternary ammonium base. Our substance does not contain aboain or kokilphin which have been found by Chen and Chen\textsuperscript{2} in their studies on the isolation of the glycosides from thevetia.

A surprising characteristic of the pharmacologic action of thevetoidin is its reversibility. This has been demonstrated in the isolated frog heart, in the failing heart of the heart-lung preparation, and in the anesthetized dog. The cumulative action later revealed in the clinical study had already been suspected following the results obtained in some of the preliminary experiments on the anesthetized dog, but had not been given the importance it deserved. The reversibility can be compared only with that described by Gold and collaborators\textsuperscript{16, 17} for acetylstrophanthin and for thevetin in their studies on the action of these substances in auricular fibrillation in the human subject. All these substances with extremely rapid action suggest more the effect of the aglycones than of the glycosides, and suggest the possibility that a carefully conducted clinical study on the aglycones might reveal actions of interest and practical importance.

The vagal effect of thevetoidin appears to us to be the most intense that has been obtained up to now with any cardiac glycoside, and in this respect and in its reversibility it resembles more the action of the aglycones and of certain alkaloids of veratrum than that of the glycosides. It is possible that this vagal effect may be responsible for the dramatic effect of thevetoidin in certain cases of paroxysmal auricular tachycardia\textsuperscript{10} and that its mechanism may consist in abolition of the abnormal automatism of the auricle by virtue of acetylcholine liberation. The fact that large doses are required to produce an intense vagal effect in the experimental animal is probably due to anesthesia which may depress the nerve centers or reflexes responsible for this action. The possibility exists that the small amounts of quaternary ammonium base detected in our samples may be in part responsible for these effects.

The dose of thevetoidin which we recommended for use in the clinic was deduced by
comparing the effects of thevetoidin with those of ouabain on the failing heart-lung preparation. It was impossible to decide on a dose by comparison of toxicity in frogs or in cats since the toxicity was about 200 times less than that of ouabain in the frog and about twenty-five times less in the cat. We have not attempted to determine the reason for this difference.

The dose of thevetoidin necessary to reverse experimental cardiac insufficiency without producing toxic effects is approximately 0.8 mg. (reservoir containing 400 cc. of blood). A therapeutic effect similar in rapidity and intensity can be obtained with 0.12 mg. of ouabain. However, this dose of ouabain causes toxic effects about one-half hour after its administration. Taking into account only the therapeutic effect, the relation of activity between ouabain and thevetoidin would be about 10 to 1, but since ouabain has a slower action than thevetoidin it is necessary to administer a larger dose in order to obtain an equally rapid effect. Experiments made with doses of ouabain less than 0.12 mg. suggested that in order to produce an effect as rapid as thevetoidin (10 to 12 minutes), it was essential to administer a dose approximately double that necessary to produce the effect of ouabain in the normal time of its action (40 to 50 minutes). This calculation would give an approximate relation of activity of ouabain to thevetoidin of 17 to 1, which approaches more closely the relation of toxicity of ouabain to thevetoidin in the cat than to the same relation in the frog. In view of these results, it was decided to begin a clinical investigation with doses of 5 to 10 mg. of thevetoidin. The dose of 10 mg. was found to be correct as may be seen in the paper dealing with the clinical aspects.10

The above results suggest the use of experimental cardiac failure in the heart-lung preparation as a method of quantitative evaluation of cardioactive substances with rapid action, above all when one is not dealing with pure glycosides but with substances containing toxic or inactive fractions which may augment or diminish toxicity in the cat. In these cases the study in experimental cardiac insufficiency by comparison with a pure glycoside of rapid action may give an indication of therapeutic activity of the substance and not of its total cardiac toxic action, which is obtained from the methods utilizing the cat and the frog.

**SUMMARY**

The method of extraction and the pharmacologic action of a crystalline substance isolated from a Mexican species of thevetia, for which the name thevetoidin is proposed, are discussed.

The digitalis-like action of thevetoidin has been demonstrated in the isolated frog heart, in the anesthetized dog and in the failing heart of the heart-lung preparation.

Thevetoidin is approximately one-twentieth as potent as ouabain. Its complete effect is exerted within about 10 minutes after its administration and disappears in approximately one hour. Its vagal action is more rapid and intense than that of those cardiac glycosides studied up to the present time.

The results indicate that the heart-lung preparation can be used for the study of the therapeutic activity of digitalis-like substances with rapid action.

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