Defibrillatory Substance from *Pteryxia terebinthina*

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A method for the isolation of a crystalline material from the roots of *Pteryxia terebinthina* has been described. The crystalline material was found to be essentially free from contractile depressant effects, and to be approximately seven times more potent than quinidine sulfate as a defibrillatory agent on isolated rat hearts.

A DESCRIPTION and the history of *Pteryxia terebinthina* (Hook.) Coult. & Rose, var. *terebinthina*, has been written by Pettinato (1) and Call (2). Call (2) obtained a crystalline material that he named pteryxin from the powdered root of this plant. A preliminary chemical and physical characterization was made by Pettinato (1) and later extended by Willette and Soine (3). Pteryxin was reported as having uterine relaxant and coronary vasodilator properties comparable to those of khellin (2, 3).

In unpublished experiments by the author crude, dealcoholized extracts of the root also demonstrated defibrillatory properties and, for this reason, it was of interest to isolate the active principle and compare its cardiac effects with those of quinidine.

EXPERIMENTAL

Extraction and Isolation

A June collection of the root was obtained from the vicinity of Moses Lake, Washington. The roots were dried and powdered in the same manner as described by Pettinato (1). A 5-Kg. quantity of the powder was macerated for 12 hours with 80% ethyl alcohol and extracted by percolation with approximately 60 L. of the menstruum. The percolate was evaporated by forced air draft until an aqueous and oily layer had separated. The aqueous layer was decanted, treated with a saturated lead subacetate solution, and filtered until no further precipitation occurred. Hydrogen sulfide was then passed through the filtrate. Excess lead, as the sulfide, was removed by filtration. The filtrate was evaporated by air to a thick syrupy consistency and partially redissolved in 95% alcohol. Insoluble material was removed by filtration. The clear liquid was stored at room temperature with access to air. Crystals appeared in the bottom of the container after 3 to 4 days. The supernatant fluid was decanted and the crystalline material redissolved in acetone with the aid of a small amount of distilled water. Partial evaporation at room temperature of this solution resulted in recrystallization of the material. Approximately 2 Gm. of crystals were obtained in this manner.

The crystals melted between 191–192°. They were very soluble in water and the solution had a weak blue fluorescence that was not apparently altered by the addition of dilute sulfuric acid nor by dilute ammonium hydroxide. No further attempt was made to determine physical or chemical characteristics.

Pharmacological Studies

Isolated Rat Heart.—Because of availability and small size, rat hearts were selected for this investigation. The procedure and apparatus for left intraventricular cannulation and coronary perfusion as described by Bryan (4) was followed. The composition of the perfusing fluid was: (Gm./L. distilled water) NaCl 7.0, KCl 0.4, $CaCl_2 \cdot 2H_2O$ 0.4, $MgCl_2 \cdot 6H_2O$ 0.2, Na_2SO_4 0.14, $NaHCO_3$ 1.5, and dextrose 1.0. The fluid was gassed with oxygen to saturation and the pH adjusted to 7.4 by means of a Beckman (model H-2) pH meter and with 5% carbon dioxide in oxygen. Perfusion pressure was approximately 40 mm. Hg and at a temperature of 37°.

Quinidine sulfate,[1] aconitine nitrate,[2] and the crystalline material that was isolated were dissolved separately in perfusion fluid for injection into the perfusion apparatus directly above the aortic cannula. Stock test solutions were prepared the day they were to be used.

Surface left ventricular electrogram was recorded through a wick electrode placed on the body at the left ventricle. Left ventricular pulse pressure (via strain gauge) was recorded with the electrogram by means of a two-channel Sanborn apparatus.

Cardioplegia.—Preliminary quantitative analysis with quinidine sulfate indicated an approximately linear relationship between dose and pulse pressure reduction that terminated in reversible cardioplegia. Prior to the isolation of the crystalline material, crude extracts of *terebinthina* also had this effect. Therefore, cardioplegia was the depressant effect used for a comparison of the two substances.

In order to test contractile depression independently from rate, hearts were driven by a Grass square wave stimulator. Rate was constant for each heart but varied between hearts.

Doses from a starting value of 100 mcg. of quinidine sulfate were increased by alternate arithmetic and geometric progression until cardioplegia was achieved. A comparable dosage schedule for the crystalline material was abandoned because of the rapid depletion of the supply, and an unexpected finding.

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[1] Merck & Co., Inc., Rahway, N. J.

[2] S. B. Penick & Co., New York, N. Y.

Fifteen hearts required an average ± S.D. of 1.72 ± 1.4 mg. of quinidine sulfate to obtain cardioplegia. In contrast, of 10 hearts, 4 showed a pulse pressure reduction of approximately 50% and 6 did not show an obvious contractile change at a dosage of 50 mg. of the crystalline material. Cardioplegia did not occur in two additional hearts with 100 mg. of the crystalline material. No further attempt was made to produce this effect and it was concluded that the crystalline material was virtually without a contractile depressant action on the rat heart.

Fibrillation.—Spontaneously beating rat hearts were used for this portion of the investigation. In order to cause fibrillation, 40 mcg. of aconitine nitrate was injected into the perfusing system. Ventricular fibrillation occurred within 1 minute, however, spontaneous reversion also occurred within approximately 5 minutes (10 hearts). Inasmuch as this unexplained circumstance was not useful, the relevance of potassium reduction in the perfusing fluid as determined by Kärki (5) was explored.

When the potassium chloride content of the perfusing fluid was reduced one-half by isosmotic sucrose substitution, aconitine-induced fibrillation was considered persistent if it lasted 15 minutes and longer. This was found to occur without exception for 10 hearts and is in agreement with Kärki whose observations were made on rate-induced fibrillation with isolated rabbit hearts. Accordingly, hearts fibrillating for 15 minutes after aconitine injection at a reduced potassium perfusing concentration were used to test defibrillation action of quinidine and the crystalline material.

Starting doses of 100 mcg. with arithmetic progression were used for quinidine sulfate, and a similar procedure starting at 10 mcg. was used for the crystalline material. To defibrillate 20/20 hearts required an average ±S.D. of 350 ± 36 mcg. of quinidine sulfate and 50.0 ± 9 mcg. of the crystalline material. At a $P = 0.05$, these means are significantly different. Therefore, it can be stated that the crystalline material possesses a potency of approximately seven times that of quinidine sulfate in its ability to defibrillate rat hearts.

Pulse pressures (reduced to essentially zero after fibrillation) were not restored to control values after defibrillation by either agent. In view of the return to control values when perfusion is continued after quinidine-induced cardioplegia, this observation was of interest. Whether aconitine or low potassium is a causative factor is not known.

In addition, when hearts in either series were defibrillated, fibrillation could not be reproduced by aconitine. This suggests a comparable, although unknown, mechanism of action for quinidine and the crystalline material.

The rat heart was selected, in part, on the premise that smaller quantities of test chemicals would be required, and it was of interest to note that the average quinidine defibrillation dose was nearly the same as that required for this purpose by isolated rabbit hearts (6). This would indicate that concentration rather than absolute amount is a critical factor in defibrillation under these conditions.

DISCUSSION

If the mechanisms for electrical and mechanical activity are considered as separate, but interrelated, then it is of obvious value to select, for defibrillatory purposes, those chemicals that have a higher degree of specificity for the former but with little or no effect on the latter. On this basis, the crystalline material isolated from *Pteryxia terebinthina* would then be preferred to quinidine. However, there are a number of other drugs and chemicals available for this purpose. Information regarding cardioplegic/defibrillatory ratios for these substances is not readily available. Therefore, no further conclusions can be reached concerning the relative potency of the crystalline material.

A second consideration—extensive information regarding *in vivo* effects of the crystalline material was not obtained. This was because of the limited quantities of the crystalline material available. In order to overcome this handicap, an attempt is being made to determine the chemical identity of the crystalline material with synthesis as an eventual objective.

CONCLUSIONS

1. A procedure for the isolation of a crystalline material from *Pteryxia terebinthina* has been described.
2. Through comparison on the isolated rat heart, the crystalline material has been shown to have a defibrillatory potency approximately seven times that of quinidine sulfate.
3. In contrast to quinidine, the crystalline material does not produce cardioplegia at a dose level of approximately 30 times that of quinidine sulfate.
4. A procedure for obtaining persistent fibrillation with aconitine in the isolated rat heart has been described.

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