Interaction of Ouabain and Malonate upon the Contractile Response of Myocardium

By DUANE G. WENZEL and IVENS A. SIEGEL

The interaction of two positive inotropic agents, ouabain and malonate, was studied upon the electrically-stimulated rat ventricle strip. Dose-response relationships of each were determined independently and in combination. The response to ouabain was linear within the concentration range tested, while malonate induced depression of the contractile force in higher concentrations. The combined administration resulted in potentiation and it was concluded that ouabain and malonate induce their positive inotropic actions through different mechanisms.

NUMEROUS attempts have been made to determine the mechanism by which the cardiac glycosides increase the efficiency of the failing heart. Such studies have formed the basis for several reviews or summaries (1–3). It has been observed that ouabain, a typical cardiac glycoside, increases the contractile force of the hypodynamic heart when glucose is employed as a substrate, yet does not affect the response in the presence of pyruvate (4). On the basis of this evidence it has been postulated that the cardiac glycosides may remove a rate-limiting step in the glycolytic pathway. Malonate, as metabolic inhibitor with positive inotropic properties, has been observed to have a similar locus of action (5). While the specific site of action within the glycolytic pathway is not known for either ouabain or malonate, it is possible to determine whether they act in the same manner by observing the nature of their interaction upon the myocardial contractile force. Such a study forms the basis of this communication.

EXPERIMENTAL

The method employed to determine the nature of the interaction between ouabain and malonate upon myocardial contractility was the electrically-driven rat ventricle strip. The details of the equipment have been described previously (6). The method, in brief, employed a strip from the right ventricle of young male rats (135–170 Gm.). Such strips were placed in a phosphate-buffered oxygenated medium containing 0.9% sodium chloride, 0.042% potassium chloride, 0.036% calcium chloride, and 1 m*M* sodium phosphate buffer at pH 7.4. The tissue was supramaximally stimulated with 20 v. at a frequency of 4 per sec. Bath volume was 100 ml. and the temperature was maintained at 27°. Drugs were added in 1 ml. of bath solution following withdrawal of an equivalent volume.

Heart strips were allowed to rest in the bath without stimulation for a period of 1.5 to 2 hours, dependent upon the time required for a satisfactory response to stimulation. Following the initiation of stimulation, during what is termed the equilibration period, the amplitude of contraction gradually increased, reaching a maximum termed the 100% level. This was then followed by a steady decline over a period of several hours. Contractile amplitude was measured at 5-min. intervals and drugs were added when it had declined to 75% of the maximal response. In this manner, tests were conducted with the heart strips being in the same relative hypodynamic state.

The method for the determination of the type of interaction between ouabain and malonate is based upon that of Loewe (7). A right isosceles triangle is formed in which the length of each leg represents the concentration of one of the pair of drugs producing the same per cent increase in contractile amplitude. If the concentration of one of the pair is reduced in a stepwise manner from the arbitrary response concentration to nothing, and if this response decrement is compensated for by necessary amounts of the other agent, the plot of the points obtained from these concentration pairs will indicate the type of interaction. If the points lie along the hypotenuse of the triangle, the drugs are additive; if they lie outside the triangle, they are antagonists; and if they lie within the triangle, the drugs are potentiative.

RESULTS

Ouabain was tested for its positive inotropic effect with seven concentrations arrayed in a geometric progression from 9 to 50 $\times 10^{-4}$ m*M*. Four strips were employed for each concentration. As shown in Fig. 1, the response was linear within this concentration range. The increase in contractile amplitude induced by ouabain reached its maximum in 5.8 $\pm$ 0.62 min. for the 28 strips used. After reaching the peak contractile force, the developed tension declined at a faster rate than that noted with untreated strips.

Twelve concentrations of disodium malonate were similarly investigated. The results are also presented in Fig. 1. Only two strips were employed with each of the larger doses of malonate, as indicated by the dotted line. The response of the strips to malonate was distinctly different from that to ouabain. In the lower concentrations (0.26–0.70 m*M*) there occurred a slight immediate decrement (1 to 4%) followed by a slow increase to slightly more than the initial 75% level after 30 to 40 min. With intermediate concentrations (1 to 5 m*M*), the initial decrement was larger (5 to 10%) and was followed by even greater increases in amplitude. The greatest positive inotropic action was developed with a concentration of 5 m*M*. At the highest con-

Received March 26, 1962, from the School of Pharmacy, University of Kansas, Lawrence.
Accepted for publication April 26, 1962.
Supported by a grant from The University of Kansas General Research Fund.

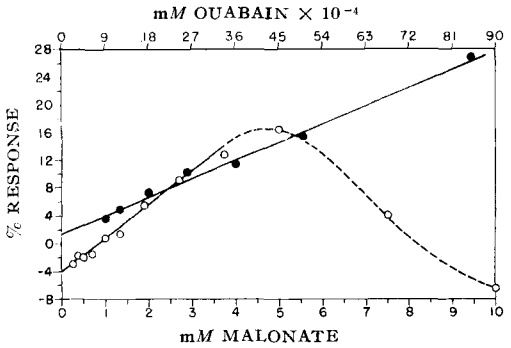

Fig. 1.—Concentration action curves of ouabain and malonate in a substrate-free medium. Ordinate: maximum per cent increase in amplitude (following an initial decrement with malonate). ●, Ouabain; ○, malonate.

centration tested (10 m*M*), a 25% decrement occurred within 1 minute and was followed by a gradual increase which never reached the original (75%) level. All per cent changes were based upon the maximum or 100% amplitude developed after the equilibration period. Those values shown in Fig. 1 are the maximum effects developed after addition of the drug without regard for the time required. With ouabain, the response was maximal in about 6 min. whereas with malonate, this maximum effect required from 30 to 40 min. In order to have the positive inotropic actions of ouabain and malonate coincide when tested for their interaction, malonate was first added to the bath and 30 minutes later, when the response was near maximal, ouabain was added.

The decision as to the per cent increase to be used was based, in part, upon the fact that malonate, in concentrations larger than 5 m*M*, exerted an inhibitory effect. If, for example, the maximum increment induced by 5 m*M* malonate (16%) were selected as the desired positive inotropic action, then any antagonism between the drugs would necessitate using inhibitory concentrations of malonate. An arbitrary 10% positive inotropic action was selected as the desired response. The concentration of each drug required to elicit this 10% positive inotropic action was determined from Fig. 1. For ouabain this was 27.5 × 10^{-4} m*M* and for malonate, 3.0 m*M*.

Five concentrations of ouabain, up to the concentration producing the 10% response (27.5 × 10^{-4} m*M*), were individually tested with sufficient malonate in order to yield the 10% increase in contractile amplitude. The five concentration combinations of ouabain and malonate with the mean values for each are presented in Table I. The curve, or isobol, formed by plotting these equieffective pairs is shown in Fig. 2. From the shape of the isobol it is apparent that the interaction is potentiative.

DISCUSSION

Ouabain was found to produce a positive inotropic action on the rat ventricle strip in concentrations as low as 9 × 10^{-4} m*M* (1:1,500,000). This was considerably below the lowest effective concentration reported by Masuoka and Saunders on the same preparation (8). As these investigators had employed a 50% decrement prior to the addition of the ouabain, in contrast to the 25% reduction in this study, the greater effectiveness could have been related to a lesser depletion of endogenous substrate. Results obtained with disodium malonate were similar to those of Covin and Berman except that they did not observe the decrease in amplitude with concentrations above 5 m*M* (5). There is no obvious explanation for such a difference in the results with high malonate concentrations.

Theoretically, there are at least several possible mechanisms by which drugs can improve the contractility of the myocardium. Energy production, transfer, or utilization may be involved. The contractile protein may be directly affected. Alterations in the electrolyte composition of the myocardium is thought by many to be the underlying mechanism. It is also possible that the efficiency with which the electrical excitation of the cell membrane is coupled with muscle response could be involved. One of the many difficulties related to studying the mode of action of positive inotropic agents is that potential mechanisms are so interdependent that an initiating event is difficult to isolate and identify. In studies employing various

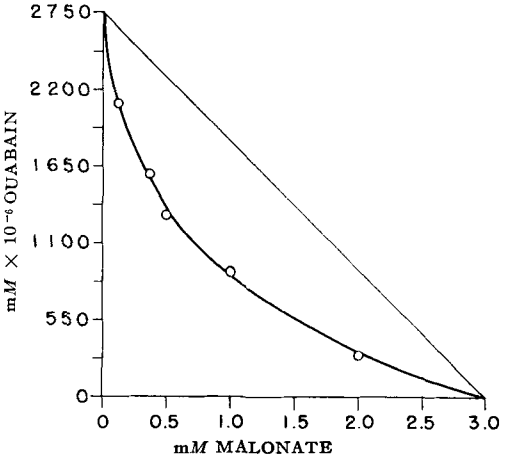

Fig. 2.—Ouabain-malonate isobol. Maximum dose of each produces a 10% increase in amplitude.

TABLE I.—CONCENTRATION PAIRS OF OUABAIN AND MALONATE PRODUCING THE SAME POSITIVE INOTROPIC ACTION[a]

	Concentration, m*M*				
Ouabain × 10^{-2}	0.21	0.16	0.13	0.09	0.03
Malonate	0.26	0.37	0.50	1.0	2.0
Mean positive inotropic action, % ± S.E.	9.99 ± 0.92	10.55 ± 1.07	10.65 ± 0.54	9.62 ± 1.14	10.58 ± 0.87

[a] Per cent positive inotropic actions are the mean values from four determinations.

substrates with a cardiac glycoside or malonate it has been demonstrated that both may act through the removal of a rate-limiting step in the conversion of glycogen or glucose to pyruvate and thus, at least in part, affect energy production or utilization (4, 5). Such an hypothesis does not account for the inhibition observed with high concentrations of malonate, but neither does it preclude the possibility of other concentration-related mechanisms. Malonate may owe a portion of its positive inotropic effect to removal of a rate-limiting step in the glycolytic pathway and possibly to its ability to function as a metabolite (9). Its inhibition of succinic dehydrogenase (10) could account for the initial decrement and that decrement observed with high concentrations. While the results of the ouabain-malonate interaction suggest neither the sites of action nor the mechanisms, they do indicate the individual actions to be different from one another. If these agents shared the same site or mechanism of action, the interaction would be either additive or antagonistic. The potentiative response indicates complementary activity.

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Stability of Vitamins A, B_1, and C in Selected Vehicle Matrices

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The relative stability of vitamins A, B_1, and C in eight commonly used solid vehicle matrices was determined. Vitamin preparations, each containing one vitamin and one solid matrix, were first subjected to accelerated aging tests and were evaluated for their loss in vitamin contents. The results indicated that a diluent moisture content in excess of one per cent played an important role in determining the stability of these preparations. Mannitol and lactose were found to be superior solid diluents on the basis of the stability studies conducted. The influence of formulation (use of coated or U.S.P.-grade vitamins), method of combination, and manufacturing procedures were studied for their effect on vitamin stability in an uncoated tablet dosage form.

IN RECENT years many new, uncoated multivitamin tablets have appeared on the market. The majority of these products have been chewable, pediatric vitamin tablets, although conventional vitamin tablet formulations in uncoated, compression-coated, and film-coated form are also making their appearance. The advantages of uncoated vitamin tablets over vitamin liquids, capsules, and coated tablets are numerous, obvious, and of considerable economic significance. The development of commercially feasible uncoated vitamin tablet formulations is being made possible by at least three advances: (*a*) the application of new excipient materials to solid vitamin products; (*b*) the commercial availability of new, more stable chemical forms of vitamins, and coated, wax imbedded, and other modifications of vitamin materials, and (*c*) studies which accurately describe the stability, reaction kinetics, and incompatibilities of the various vitamins.

Received March 29, 1962, from Purdue University, School of Pharmacy, Lafayette, Ind.
Accepted for publication May 24, 1962.
Abstracted from a thesis submitted to the Graduate School of Purdue University, by K. N. Wai in partial fulfillment of the requirements for the Master of Science degree.
Presented to the Scientific Section, A.PH.A. Las Vegas meeting, March 1962.

The reports of McLaughlan, Clark, Campbell, and McLeod (1–3) have revealed that vitamins A, B_1, B_{12}, and pantothenic acid in many commercial multivitamin products are quite unstable. Ascorbic acid is known to be incompatible with riboflavin, vitamin B_{12}, and folic acid (4). Thiamine reacts with riboflavin (5) and folic acid (6). Extensive investigations of the interactions among thiamine, niacinamide, and vitamin B_{12} have also been carried out in the past few years (7–14).

Accelerated aging tests of vitamin products have been conducted by Garrett (15) and Yamamoto (16). These workers related the stability of various vitamins with storage time and temperature and found that the degradation of ascorbic acid, thiamine, vitamin B_{12}, and pantothenyl alcohol was a first-order reaction and the degradation rate of vitamin A was independent of concentration. They also showed that at 45° the degradation of vitamin A in 8 weeks and ascorbic acid in 6 weeks was approximately equivalent to their degradation at room temperature for 1 year. Taub and Katz (17) have indicated that thiamine decomposition in tablets and capsules, after storage at 45°