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The actions of dehydroemetine on isolated guinea-pig atria. Influence of ouabain and calcium

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Summary

1. The mechanism of the inhibitory effect of dehydroemetine on the heart was investigated using (a) the spontaneously contracting isolated guinea-pig atrial preparation and (b), the electrically driven left atrial preparation.

2. Dehydroemetine decreased the rate and amplitude of contraction of spontaneously contracting atria.

3. Increasing the calcium concentration of the bath fluid to 28·2 mM abolished the effect of dehydroemetine on amplitude of contraction while the effect on rate persisted.

4. Pretreatment with dehydroemetine increased the capacity of ouabain to induce arrhythmias in spontaneously contracting atria. Similarly pretreatment with ouabain augmented the inhibitory effect of dehydroemetine on spontaneous atrial activity.

5. In the electrically driven left atrial preparation, dehydroemetine caused a decrease in contractile strength which was opposed by ouabain and by increased calcium concentration of the bath fluid.

6. The effect of dehydroemetine on spontaneously contracting and on electrically driven atria was mimicked by adding excess potassium to the bath fluid.

7. It was concluded that the effects of dehydroemetine on rate and contractility are, to a large extent, independent of one another. The effect on spontaneous rate is consistent with alteration of the potassium permeability of the myocardial cell membrane while the effect on contractility appears to be due to a decrease in the uptake of calcium from the medium by the myocardial cell.

Introduction

In an earlier communication, Durotoye & Salako (1972) showed that dehydroemetine caused a reduction in the spontaneous frequency of contraction of isolated guinea-pig atria and evidence was adduced to suggest that this effect might be due to an increase in potassium permeability of the myocardial cell membrane. Alterations in the strength of contraction of spontaneously beating atria were also produced by concentrations of dehydroemetine which had a negative chronotropic effect. However, since contractile force of the atrial myocardium is dependent on the rate of contraction (Koch-Weser & Blinks, 1963), the spontaneously contracting atrial preparation could not be used to study the inotropic effect of the drug in any detail. The purpose of the present study was (1) to obtain further information on the mechanism of the negative chronotropic effect of dehydroemetine by studying its

interactions with ouabain and calcium in the spontaneously contracting atria and (2) to study the effect of the drug on the force of myocardial contraction by using isolated left atria maintained at a constant frequency by electrical stimulation.

Methods

Guinea-pigs of either sex weighing between 400 and 600 g were used. The animals were killed by a blow on the head followed by exanguination. The whole heart was removed and the ventricles discarded. For studies on pacemaker activity, both atria were suspended vertically in one piece in a 70 ml isolated organ bath. One end was attached to a hook at the bottom of the bath and the other was attached via a fine silk thread to a light Starling's heart lever exerting a tension of approximately 1 gramme. Spontaneous isotonic contractions of the atria were recorded with 7-fold magnification on smoked paper. The preparation was allowed to equilibrate in the bath fluid for 90 min before experiments commenced.

Effects on force of contraction were studied using left atrial preparations. These were obtained by carefully cutting along the interatrial septum and discarding the right atrium. The separated tissue was tied at one end to a pair of bipolar platinum electrodes at the bottom of a 70 ml organ bath. The upper end was attached via a fine silk thread to a Statham force-displacement transducer with a resting tension of 0·8–1 gramme. The tissue was stimulated with supramaximal rectangular pulses (20–50 V) of 1·5 ms duration, and the isometric contractions were recorded on a Schwarzer physiograph (Schwarzer Ltd., Munchen, W. Germany). An interval of 30 min was allowed between setting up the preparation and commencing electrical stimulation. Spontaneous activity, usually present when the atrium was first set up, stopped, as a rule, during this interval. The atrium was stimulated for 60 min before experimental tests were begun. The frequency of stimulation used throughout the study was 1·5 Hz except in experiments in which the effect of change in frequency was tested when another frequency, 0·15 Hz, was also used. After changing to the lower frequency, stimulation was maintained until the tension became steady before taking any measurements. The routine frequency of 1·5 Hz was chosen because it approximated most closely to the spontaneous rate recorded in experiments with whole atria.

The tissues were immersed in Locke solution with double glucose of the following composition (mM): sodium chloride 153·8, potassium chloride 5·6, calcium chloride 2·2, sodium bicarbonate 5·95, glucose 11·1. The bath fluid was maintained at 30° C and was continuously bubbled with 100% oxygen.

Drugs and chemical compounds were added to the bath fluid to obtain the desired concentration. After each addition, the tissue was allowed to attain a steady state before measurements were made. Dose-response curves for dehydroemetine were determined by adding the drug cumulatively to the same tissue. With other compounds, different concentrations were tested on different atria. In all cases, although the effects were usually completely reversible by washing, each tissue was used for only one experiment.

The drugs used were dehydroemetine dihydrochloride (Roche) and ouabain (British Drug Houses). Concentrations were expressed as μg/ml of the salt. Concentrations of potassium and calcium in the bath fluid were varied by changing the concentrations of potassium chloride and calcium chloride respectively. In-

creases in the concentrations of these cations were produced by adding appropriate volumes of 1·4 molar solution to 70 ml of the standard Locke solution in the bath. Osmotic balance was not attempted in either case.

Results presented in the Tables and Figures are mean values ± standard errors of the means. Comparisons of results were made using Student's *t* test.

Results

(a) Spontaneously contracting atria

Dehydroemetine caused a reduction in the rate and amplitude of spontaneous contraction of isolated guinea-pig atria. The effect was dose-related, 0·15 μg/ml being the minimal concentration required to produce an effect while 15 μg/ml regularly caused cessation of spontaneous activity. The onset of the effect was immediate but the subsequent time course was gradual. With concentrations that did not arrest spontaneous contraction, new steady levels were reached between 5 and 20 minutes. A concentration of 15 μg/ml which caused cessation of beat usually did so within 15 minutes; the effect was reversible by washing.

Interaction with ouabain. The influence of ouabain on the action of dehydroemetine was studied using two concentrations of ouabain, 0·125 and 0·375 μg/ml. Initial experiments showed that both concentrations of ouabain markedly increased the amplitude of spontaneous contraction of the atria with minimal increases in rate (0–8%) during an observation period of 60–90 minutes. A concentration of 1 μg/ml, also tested during these initial experiments, caused arrhythmias in 8 out of 11 experiments. This concentration was therefore considered 'toxic' under the experimental conditions and was no longer used.

In one series of experiments, the effects of the two concentrations of ouabain were tested in atria pretreated for 30 min with different concentrations of dehydroemetine. Ouabain had no effect on atria after the spontaneous contractions had been arrested by dehydroemetine, 15 μg/ml. In atria partially inhibited by dehydroemetine, 1·5 μg/ml, the small positive chronotropic effect of ouabain was converted to a negative chronotropic effect (Fig. 1). In addition, ouabain 0·375 μg/ml, produced arrhythmias in 4 out of the 19 atria pretreated with dehydroemetine, 1·5 μg/ml, while ouabain, 0·125 μg/ml, caused arrhythmias in a further two atria. These six experiments were not included in the results summarized in Fig. 1.

In another series of experiments, the effects of different concentrations of dehydroemetine were tested cumulatively in atria pretreated for 60 min with ouabain and the results compared with the effects of dehydroemetine in untreated atria. In both ouabain-treated and untreated atria, dehydroemetine 15 μg/ml, caused complete arrest of spontaneous atrial contraction. The remaining results are summarized in Table 1. Treatment with dehydroemetine 0·15 and 1·5 μg/ml, produced a greater negative chronotropic effect in ouabain-treated than in untreated atria; additionally, dehydroemetine, 1·5 μg/ml, caused cessation of spontaneous contraction in a substantial proportion of atria pretreated with ouabain but in none of the untreated atria. The cessation of beat produced in ouabain-treated atria was usually abrupt, preceded only by a moderate reduction and an irregularity in rate and amplitude, in contrast to the gradually progressive inhibition produced concentrations of 15 μg/ml or higher in untreated atria (Durotoye & Salako, 1972).

Interaction with calcium. Addition of calcium chloride to the bath fluid to obtain

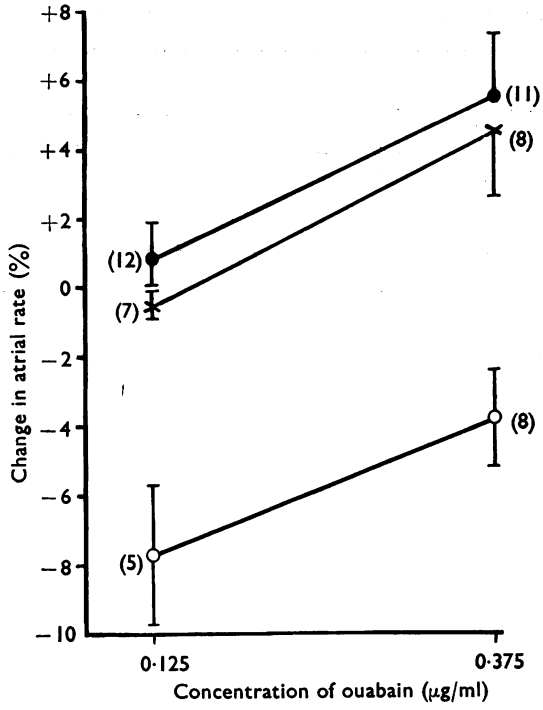

FIG. 1. Influence of different concentrations of dehydroemetine on the chronotropic effect of ouabain on spontaneously contracting guinea-pig atria. ●, No dehydroemetine; ×, ○, atria pretreated with 0·15 and 1·5 μg/ml dehydroemetine respectively. Numbers of experiments are given in parentheses. The points represent the percentage change (mean ± S.E.) from the values before the addition of ouabain.

calcium concentrations of 4·2, 12·2, 22·2 or 28·2 mM increased amplitude of contraction without affecting rate. In atria completely inhibited by dehydroemetine, 15 μg/ml, automaticity was restored by 28·2 mM calcium but not by the lower concentrations. Partial atrial inhibition by dehydroemetine, 0·15 and 1·5 μg/ml, was antagonized by raising the external calcium concentration. Addition of calcium chloride to the medium in such partially inhibited atria always caused an increase in the amplitude of contraction but the effect on rate was variable. When the rate was markedly reduced by dehydroemetine (e.g. to less than 25% of the control rate), an increase in rate occurred following addition of calcium, otherwise the rate remained unchanged (Fig. 2). In another series of experiments, addition of dehydroemetine, 15 μg/ml, to atria previously contracting in solution containing

TABLE 1. *Effect of different concentrations of dehydroemetine on spontaneous frequency of control and ouabain-treated guinea-pig atria*

Concentration of dehydroemetine (μg/ml)	Pretreatment	Number of atria tested	Number giving 100% inhibition	% Reduction in partially inhibited atria. Mean and (range)
0·15	Ouabain 0·375 μg/ml	11	Nil	4·2 (0–23·3)
0·15	Ouabain 0·125 μg/ml	14	Nil	3·1 (0–9·1)
0·15	Nil	15	Nil	1·5 (0–3·3)
1·5	Ouabain 0·375 μg/ml	11	6	19·4 (6·7–55·0)
1·5	Ouabain 0·125 μg/ml	14	5	16·3 (3·3–55·7)
1·5	Nil	18	Nil	9·9 (5·0–13·6)

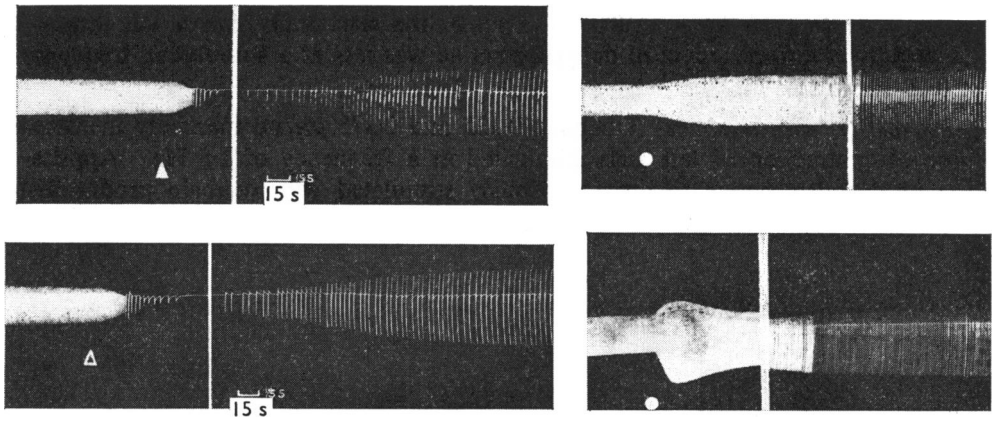

FIG. 2. Records from four spontaneously contracting isolated guinea-pig atria preparations. Top row, first and second panels: effect of 28·2 mM calcium (added between the panels) after completely inhibiting the atria with 15 μg/ml dehydroemetine added at ▲. Top row, third and fourth panels: effect of 15 μg/ml dehydroemetine (added between the two records) on atria previously stimulated with 28·2 mM calcium added at ●. Bottom row, first and second panels: reversal of the effect of 15·6 mM potassium added at △ by 28·2 mM calcium, added between the panels. Bottom row, third and fourth panels: 28·2 mM calcium was added at ●, and followed by 15·6 mM potassium between the two records.

28·2 mM calcium caused a reduction in rate but not in amplitude of contraction (Fig. 2).

Effect of potassium excess. Addition of potassium chloride to the bath fluid, to obtain potassium concentration of 13–25 mM, produced a progressive reduction in the rate and amplitude of contraction of the atria until spontaneous activity finally stopped. Ouabain, 0·125 and 0·375 μg/ml, failed to restore spontaneous activity but this was readily restored by 28·2 mM calcium. Raising the potassium concentration of the bath fluid to 13 mM or greater also caused complete inhibition of atria pretreated with ouabain, 0·125 and 0·375 μg/ml. On the other hand, these concentrations of potassium caused only a reduction in rate, without affecting the amplitude of contraction, of atria suspended in a solution containing 28·2 mM calcium (Fig. 2). In all these respects, the effects of excess potassium in the bath fluid closely mimicked that of dehydroemetine.

(b) Electrically driven left atrium

Dehydroemetine caused a dose-dependent reduction in the force of contraction of isolated left atria stimulated at a frequency of 1·5 Hz. This negative inotropic effect was comparatively less than the effect on pacemaker activity. Thus dehydroemetine, 15 μg/ml, which regularly abolished spontaneous activity, caused only

TABLE 2. *Effect of frequency of stimulation on the negative inotropic effect of dehydroemetine* (15 μg/ml) *on electrically driven left atria*

Stimulation rate (Hz)	Tension developed (g) Before dehydroemetine	After dehydroemetine	% Reduction in tension by dehydroemetine
0·15	0·226±0·019	0·159±0·022	29·6±4·49*
1·5	0·929±0·052	0·407±0·030	56·2±2·01*

* Difference between these two values is highly significant ($P<0·01$). Values given are means±S.E. of 13 experiments.

partial reduction of the contractile tension of the electrically driven left atrium; the negative inotropic effect of dehydroemetine was less at a stimulation frequency of 0·15 Hz (Table 2).

Interaction with ouabain. Ouabain, 0·125 and 0·375 μg/ml markedly increased force of contraction of left atria stimulated at a frequency of 1·5 Hz. Application of dehydroemetine to atria previously stimulated with ouabain produced a negative inotropic effect which was less than the effect on untreated atria (Fig. 3).

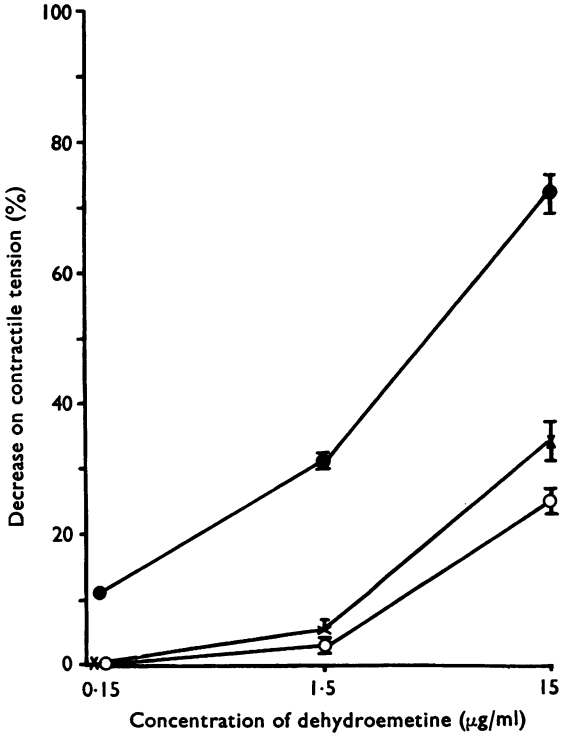

FIG. 3. Effect of different concentrations of dehydroemetine on contractility of isolated left atria stimulated at a frequency of 1·5 Hz in the presence and absence of ouabain. ●, No ouabain; ×, ○, atria pretreated with 0·125 and 0·375 μg/ml ouabain respectively. Each point represents the mean ± S.E. of six experiments.

Conversely, pretreatment with dehydroemetine reduced the magnitude of the positive inotropic effect of ouabain, the lower dose of ouabain being more readily antagonized than the higher dose (Fig. 4).

Interaction with calcium. Addition of calcium chloride to the bath fluid to obtain calcium concentrations of 4·2, 12·2 and 28·2 mM markedly increased contractile tension of left atria, stimulated at a rate of 1·5 Hz. With 28·2 mM calcium in the external medium, the negative inotropic effect of dehydroemetine, 15 μg/ml, was abolished. With external calcium concentrations of 2·2, 4·2 and 12·2 mM the percentage inhibitions of contractile tension by dehydroemetine, 15 μg/ml, were 68·7 ± 4·6, 44·8 ± 1·9 and 21·4 ± 2·5 (means ± S.E. of 6 experiments) respectively. Similarly when excess calcium was added to the bath fluid in atria pretreated with dehydroemetine, 15 μg/ml, there was a reduced response to 4·2

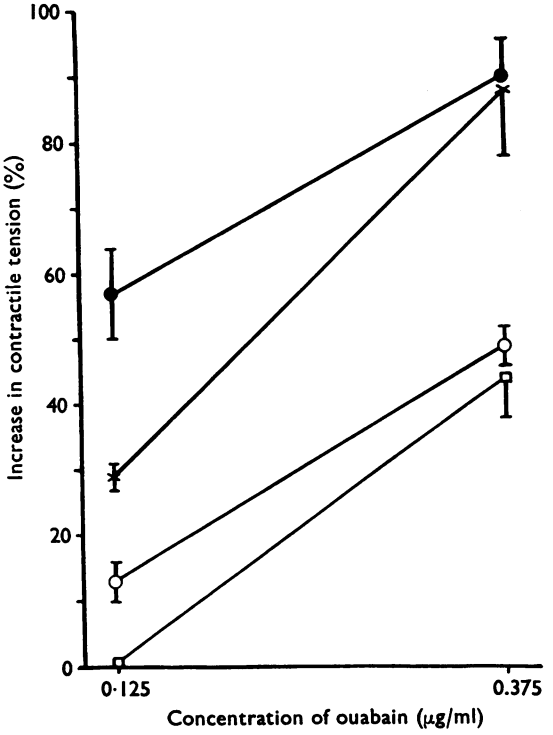

FIG. 4. Effect of ouabain on the contractile force of electrically driven left atria in the presence and absence of dehydroemetine. ●, No dehydroemetine, ×, ○, □, atria pretreated with 0·15, 1·5 and 15 μg/ml dehydroemetine respectively. Each point represents the mean ± S.E. of six experiments.

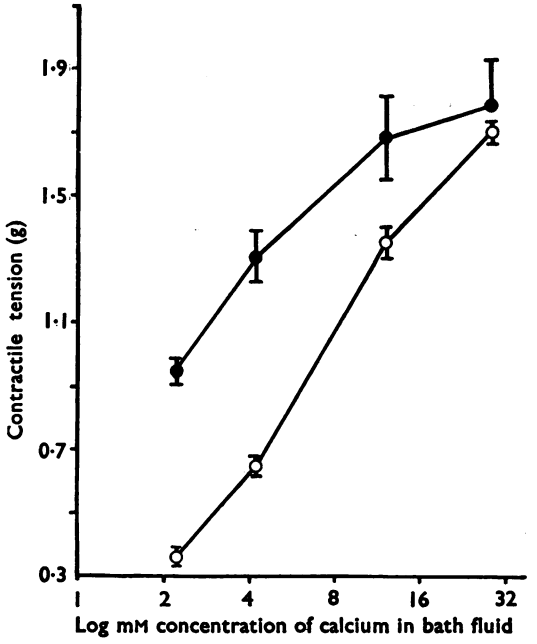

FIG. 5. Effect of varying the concentration of calcium in the bath fluid on the contractile tension of electrically driven left atria in the absence (●) and presence (○) of 15 μg/ml dehydroemetine. Each point represents the mean ± S.E. of six experiments.

mM calcium but the tension developed approached that of untreated atria as the external calcium concentration was further increased (Fig. 5).

Effect of excess potassium in the external medium. Addition of potassium chloride to the bath fluid to obtain a potassium concentration of 15·6 mM caused a reduction in the force of atrial contraction which was significantly less in the presence of ouabain (0·375 μg/ml) or raised calcium concentration (28·2 mM) in the external medium than in untreated atria. Thus, in untreated atria, the percentage inhibition of contractile strength was $77{\cdot}9 \pm 2{\cdot}2$ compared with $51{\cdot}9 \pm 3{\cdot}5$ and $11{\cdot}6 \pm 2{\cdot}0$ in ouabain and calcium-treated atria respectively (means $\pm$ S.E. of 6 experiments). Similarly in the presence of 15·6 mM potassium concentration in the bath fluid, the positive inotropic effect of ouabain, 0·375 μg/ml, was significantly reduced ($21{\cdot}2 \pm 3{\cdot}5$ percentage increase compared with $90{\cdot}4 \pm 5{\cdot}6\%$ in control experiments. $n=6$). The positive inotropic effect of raising the external calcium concentration to 4·2 mM was also reduced by suspending the atrium in bath fluid containing 15·6 mM potassium but, as in the case of dehydroemetine-treated atria (see Fig. 5), this antagonism was overcome by increasing the external calcium concentration to 28·2 mM (Fig. 6) so that the tension developed in 28·2 mM external calcium was not significantly different whether the atrium was suspended in standard Locke solution or in Locke solution containing 15·6 mM potassium.

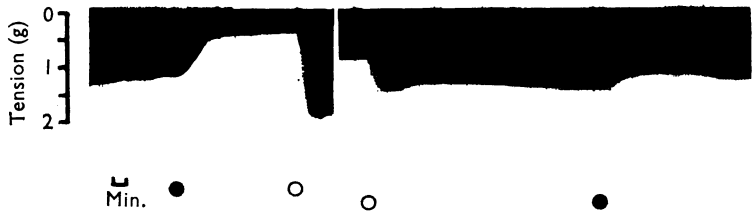

FIG. 6. Two isolated left atrium preparations stimulated at afrequency of 1·5 Hz. Left hand panel: at ● potassium concentration of the medium was raised to 15·6 mM, and at ○, calcium concentration of the medium was increased to 28·2 mM. Right hand panel: at ○, calcium concentration of the medium was raised to 28·2 mM, followed at ●, by increasing the medium potassium concentration to 15·6 mM. Vertical calibration in grammes. Contraction downward.

Discussion

Spontaneously contracting atria

The results confirm the previous findings of Durotoye & Salako (1972) that dehydroemetine inhibits the pacemaker activity of isolated spontaneously contracting atria, the effect being similar to that induced by high concentrations of potassium in the bath fluid. It was suggested by these workers that the effect might be due to an increase in the permeability of the myocardial cell membrane to potassium. Such an action would cause an increase in potassium efflux during the repolarization and the diastolic depolarization phases of the action potential. This initially would cause hyperpolarization of the membrane with slowing of the spontaneous rate (Toda & West, 1967). However, continued loss of potassium from the intracellular to the extracellular space would lead to a progressive diminution of the ratio $[K_i]/[K_o]$ on which the resting membrane potential of the cell largely depends (Shanes, 1958). Complete cessation of spontaneous contraction would thus be due to persistent depolarization of the membrane in much the same way as that caused by excess potassium in the bath fluid (Shanes, 1958). A loss of about 10% of intra-

cellular potassium has been associated with cessation of spontaneous activity due to persistent depolarization (Rayner & Weatherall, 1957).

It is known that excess calcium concentration in the medium can repolarize cardiac tissue depolarized by excess potassium and thus restore spontaneous activity (Walker & Weatherall, 1964). The ability of increased calcium concentration of the external medium to restore automaticity in atria made quiescent by a high external potassium concentration has been confirmed in this study and the similarity of the effect of dehydroemetine with that of excess potassium in the medium was further demonstrated by the ability of excess calcium to restore automaticity in atria arrested by dehydroemetine.

Atria pretreated with dehydroemetine are readily made arrhythmic by 'non-toxic' doses of ouabain. 'Toxic' concentrations of ouabain are thought to induce arrhythmias by inhibiting the sodium-for-potassium active transport mechanism across the myocardial cell membrane. As a result of this, the cells gain sodium and lose potassium. The membrane potential becomes less negative and nearer the critical level for spontaneous depolarization. This could lead to increased excitability with arrhythmia (Weatherall, 1966). Since the suggested action of dehydroemetine would also lead to a loss of intracellular potassium, it is not surprising that its combination with ouabain increases the toxicity of the latter and vice versa. By contrast, increased potassium concentration of the external medium reduces the capacity of ouabain to produce cardiac arrhythmia (Baker, 1947). This is due to the fact that increased external potassium concentration, while decreasing the ratio $[K_i]/[K_o]$ tends to cause an increase in intracellular potassium (Walker & Weatherall, 1964), and thus oppose the action of ouabain that leads to arrhythmia. The effects of dehydroemetine are therefore not mimicked in every respect by excess medium potassium.

In atria stimulated by increasing the calcium concentration of the bath fluid from 2·2 to 28·2 mM, dehydroemetine caused a fall in the rate but not in the amplitude of contraction suggesting that the two effects of dehydroemetine can be separated. A similar dissociation of the negative inotropic from the negative chronotropic effects of excess potassium was produced by pretreatment with calcium chloride which is a further evidence of the similarity of the actions of dehydroemetine and excess potassium in the external medium.

Electrically driven left atria

Dehydroemetine decreased the force of contraction of electrically driven left atria. The observation that a concentration of dehydroemetine which regularly abolished spontaneous rate produced only partial inhibition of force of contraction further suggests that the two effects are, at least partially, independent of one another.

The negative inotropic effect of dehydroemetine was reduced by decreasing the stimulation rate from 1·5 to 0·15 Hz and by pretreatment with ouabain or excess calcium in the medium. It is now generally agreed that myocardial contraction is associated with uptake of calcium by the contractile elements within the myocardial cell (Koch-Weser & Blinks, 1963). Dehydroemetine would be expected to inhibit the force of myocardial contraction if it interfered, in some way, with the calcium uptake mechanism. Teiger & Farah (1967) using the isolated left atrial preparation

of the rabbit demonstrated a rapidly exchangeable tissue-calcium component in which the rate of calcium exchange increased with increasing rates of stimulation. The greater effect of dehydroemetine at the faster frequency could thus be explained on the basis that a rapid calcium turnover rate is more susceptible to inhibition than a slower one. It has also been shown (DiPalma & Mascatello, 1951) that the refractory period of isolated cardiac muscle is inversely related to the frequency of contraction, and Benforado (1958) proposed that a drug which produces an increase in the effective refractory period acts more quickly and strongly as the refractory period decreases, that is, as frequency increases. Since dehydroemetine increases the effective refractory period of guinea-pig atria (Durotoye & Salako, 1971), it might also be expected, on the basis of the foregoing, to produce a greater effect on contractile force at higher stimulation frequency.

It has been shown by several workers that ouabain and other cardiac glycosides cause accumulation of calcium within the myocardial cell (Sekul & Holland, 1960; Thomas, 1960a; Lüllmann & Holland, 1962). The inotropic effect of cardiac glycosides is therefore thought to result from an increase in the amount of calcium available for coupling to the contractile elements within the myocardium during systole. If the action of dehydroemetine on force of contraction arises from interference with calcium uptake by the cell, then the prior accumulation of calcium within the cell under the influence of ouabain would have an antagonizing effect on dehydroemetine. Similarly prior administration of dehydroemetine, by interfering with the uptake of calcium into the myocardial cell, will inhibit the rate of intracellular accumulation of calcium induced by ouabain and so reduce the positive inotropic effect of the drug. Like ouabain, increasing the calcium concentration of the bath fluid causes a marked increase in the calcium content of the myocardial cell (Teiger & Farah, 1967). It is therefore not surprising that interactions between ouabain and dehydroemetine and between calcium and dehydroemetine are similar.

In the stimulated left atrial preparation, as in the spontaneously contracting atria, the effect of dehydroemetine is mimicked by increasing the potassium concentration of the bath fluid. Lowering the potassium concentration of the external medium increases calcium uptake by the myocardial cell (Thomas, 1960b), while increasing the potassium concentration of the medium releases calcium from its sites of accumulation in the myocardium (Walker & Weatherall, 1964). Hence the effects of external potassium concentration on strength of contraction are ultimately linked with calcium uptake and release. It is therefore not surprising that the negative inotropic effect of excess external potassium is antagonized by ouabain and by increasing the calcium concentration of the bath fluid.

In conclusion, the present experiments have shown that the negative chronotropic and the negative inotropic effects of dehydroemetine on the isolated guinea-pig atria are probably independent of one another. The results support the suggestion from the earlier studies of Durotoye & Salako (1972) that the effect of dehydroemetine on spontaneous atrial rate is due to an increase in the permeability of the myocardial cell membrane to potassium which allows the leakage of potassium from the intracellular to the extracellular space. From the results of the present studies on electrically driven left atria it would appear that dehydroemetine can also interfere with the uptake of calcium by the myocardial cell and thereby inhibit its contractility. Such an effect could be due to a decrease in the permeability of the myocardial cell membrane to calcium. A similar effect would also be pro-

duced if dehydroemetine has a chelating action on calcium but this type of action has not so far been demonstrated for the drug. The readiness with which the effects of dehydroemetine are reversed by washing and by changing the external ionic concentration makes it unlikely that the effects result from derangement of essential intracellular metabolic activities. The similarity between the effect of dehydroemetine and that of increased external potassium concentration on atrial contractility can be explained on the basis that both actions have a common final pathway which is to diminish the amount of calcium available for coupling to the contractile elements during systole. Also, in view of the similarity of the effects of the two compounds and the fact that alterations in the potassium concentration of the medium affects the calcium content of the myocardium, it is possible that the effect of dehydroemetine on contractility (and hence on calcium uptake) is linked at some stage, to its suggested action on potassium movement across the myocardial cell membrane. Finally, because of the nature of the experiments, these conclusions can at present only be tentative and verification must await studies on potassium and calcium fluxes and uptake in myocardial tissue under the influence of dehydroemetine.

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