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THE EFFECTS OF HEART RATE ON THE ACTION POTENTIAL OF GUINEA-PIG AND HUMAN VENTRICULAR MUSCLE

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SUMMARY

1. On increasing the stimulation frequency of isolated pieces of guinea-pig ventricular muscle, the resting potential depolarizes, and the action potential duration and amplitude are reduced. On termination of the high frequency train of action potentials, these changes are reversed.

2. The resting potential changes are roughly exponential, with a time constant of the order of 10 sec, and are attributable to K^+ accumulation in the extracellular space. They are not explicable in terms of known gating variables.

3. The action potential duration and amplitude recover much more slowly than the resting potential, after a high frequency train (half-time $\sim$ 5 min). The time course of these recoveries is not exponential, and is slower after trains which produce more shortening of the action potential. The slow time course suggests that K^+ accumulation is not the main cause of the changes in action potential shape. Furthermore, when a certain depolarization of the resting potential is produced by a high frequency train, there is a greater reduction of the action potential duration than that which occurs when the bathing $[K^+]$ is raised to produce the same depolarization (Reiter & Stickel, 1968). This is so even when a gradient of extracellular $[K^+]$ is induced in the preparation, to mimic non-uniform K^+ accumulation.

4. Similarly, the shortening of the action potential produced by toxic doses of cardiotonic steroids is probably not the result of K^+ accumulation.

5. The slow changes of the action potential shape produced by a high frequency train are not attributable to the effects of gating variables, nor (solely) to a rise in the intracellular Na concentration stimulating the electrogenic Na/K pump. The dye 3,3′-diethylthiadicarbocyanine, which blocks the Ca^{2+}-activated K conductance in the erythrocyte, has no significant effect on the shape changes.

6. After a sudden change in heart rate, the QT interval of the human electrocardiogram (e.c.g.) changes slowly to a new equilibrium value. The time course of this change is similar to that of the action potential duration in guinea-pig ventricle following a change in stimulation frequency. These changes of the e.c.g. are probably not due to slow alterations of neural or hormonal factors extrinsic to the heart. In the whole heart, the effects on the ventricular action potential duration of changes

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in sympathetic or vagal tone, or of circulating catecholamines, can be largely accounted for by the changes of atrial driving frequency they produce.

INTRODUCTION

Voltage-clamp studies have allowed the characterization of gated currents in several cardiac preparations, and these currents can account for the basic properties of the cardiac action potential (McAllister, Noble & Tsien, 1975; Beeler & Reuter, 1977). However, the changes of the action potential which occur following a change in heart rate, or on application of cardiac glycosides, cannot be explained in terms of the known gating properties of these currents (Reiter & Stickel, 1968; Cohen, Daut & Noble, 1976*a*).

Recent voltage-clamp studies have emphasized that, in addition to gating changes, there are changes in intra- and extracellular ion concentrations which contribute to the time dependence of the membrane current (Bassingthwaite, Fry & McGuigan, 1976; Maughan, 1973; Noble, 1976; Baumgarten & Isenberg, 1977). Accumulation of K^+ ions in the extracellular spaces during depolarizing clamp pulses has been demonstrated with a [K^+]-sensitive electrode (Cleeman & Morad, 1976), and is significant even during a single action potential (Kline & Morad, 1976). Such accumulation has been suggested to be partly responsible for the repolarization phase of the action potential (Weidmann, 1956; Hoffman, 1972), and to be the mechanism of action potential shortening produced by toxic doses of cardiotonic steroids (Cohen *et al.* 1976*a*; Hoffman, 1972; Miura & Rosen, 1978).

In this paper we examine whether K^+ accumulation can account for the effects of heart rate changes and cardiotonic steroid intoxication on the ventricular action potential. Our procedure was to study changes in the action potential shape, using the resting potential as an approximate index of K^+ accumulation. This approach has serious limitations, but it may be as informative as using the voltage-clamp technique. The latter is most powerful when the membrane current depends only on voltage and time: if K^+ accumulation occurs, the current time course depends on the history of the current flowing, and there is no general way to separate the contributions of gating and accumulation changes to the voltage-clamped current (Attwell, Eisner & Cohen, 1979*b*).

The results suggest that, although K^+ accumulation is one determinant of the ventricular action potential shape, there is another more slowly changing factor which is usually dominant (also see Carmeliet, 1958; Boyett & Jewell, 1978).

A preliminary report of these results has been presented (Attwell, Cohen, Eisner & Noble, 1977).

METHODS

Guinea-pig hearts were obtained from animals killed by a blow to the back of the neck. The ventricles were immediately opened and small slices, 1 mm thick and 2 mm in diameter, were removed from the endocardial septal area. We chose such preparations, rather than the more conventional papillary muscle, since the former contracted less vigorously and allowed more stable electrical recording.

The electrical recording had to satisfy two criteria. First, the resting potential in the absence of stimulation had to be stable to within 0·5 mV over a period of several minutes, so that small depolarizations could be measured after an increase in stimulation frequency. Secondly, several experiments, such as that in Fig. 12, lasted up to 7 hr, in order to examine stimulation periods of

different frequency and duration. It was important that the electrode remained in one cell for the whole experiment. Furthermore, we always checked that the control action potential duration and amplitude (at 0·5 Hz stimulation frequency) did not change by more than 10% during such a long experiment. We generally left the preparation to recover for 1–2 hr after dissection. Under these conditions the preparation was subsequently sufficiently stable for several hours.

The majority of the experiments were performed in Tyrode solution containing (mM): NaCl 128; $NaHCO_3$ 12; NaH_2PO_4 0·4; $MgCl_2$ 1; $CaCl_2$ 2; and glucose 2 g/l. The KCl concentration was varied according to the experiment, and is stated in the text. The pH was 7·2–7·4 and the solution was bubbled continuously with 95% O_2/5% CO_2. In some experiments a Tris buffer were used: this solution contained (mM):NaCl 140; $MgCl_2$ 1; $CaCl_2$ 2; Tris 10; and glucose 2 g/l. This solution was bubbled with 100% O_2, and the pH was 7·2–7·4. No difference was detected between the results obtained in the two solutions. The temperature was controlled by a Peltier element and feed-back system. Unless otherwise stated, the temperature was maintained to within 0·2 °C of a value between 33 and 38 °C.

Human data were obtained during routine clinical investigations made for therapeutic and diagnostic purposes, in collaboration with Dr D. Bennett of the Radcliffe Infirmary, Oxford. Right atrial pacing was carried out via trans-venous cannulae. Conventional three-lead e.c.g. recording (leads 1, V1, V6) was used from the surface of the body. Changes in QT interval were measured using the lead pair showing the most clearly defined T wave. In the particular patients that we obtained data from, the clinical procedure being used was to apply high frequency pacing and then to stop pacing abruptly, allowing the heart rate to attain whatever value naturally occurred.

RESULTS

Guinea-pig studies

Resting potential changes during activity

Fig. 1*A* shows the effect of 5 Hz trains of action potentials on the resting potential of guinea-pig ventricular muscle. The maximum diastolic potential (initially −74 mV) depolarizes during the train, reaching a maximum depolarization (after long trains) of 12 mV. The depolarization decays after the train, with a half-time of ~ 10 seconds. Time-dependent currents found so far in the ventricle have time constants of less than 500 msec in the diastolic potential range (Katzung & Morgenstern, 1977). so these changes of the maximum diastolic potential are not due to known gating changes. In other preparations, $[K^+]$-sensitive micro-electrodes have been used to show that the depolarization of the maximum diastolic potential produced by a train of action potentials is associated with a rise in the extracellular K^+ concentration (Kline & Morad, 1976; Kunze, 1977). Similar experiments in our guinea-pig ventricle preparation have confirmed that K^+ accumulation occurs there too and decays over a period similar to that of the maximum diastolic potential changes (S. Barton, I. Cohen & R. Kline, unpublished). We attribute the depolarization that we observe to K^+ accumulation, therefore, since the resting potential is reasonably sensitive to the extracellular $[K^+]$ in this potential range (Fig. 1*B*).

The recovery of the resting potential

Fig. 2 shows semilogarithmic plots of the resting potential after impulse trains of different lengths in one preparation. Apart from the first second after the train, the recoveries can be approximately fit by a single exponential. The recovery of the resting potential does not depend significantly on the train length (see Fig. 5*A*).

The recovery of the resting potential is expected to depend partly on the rate at which K^+ is pumped into the cells. To investigate this, we reduced pump activity

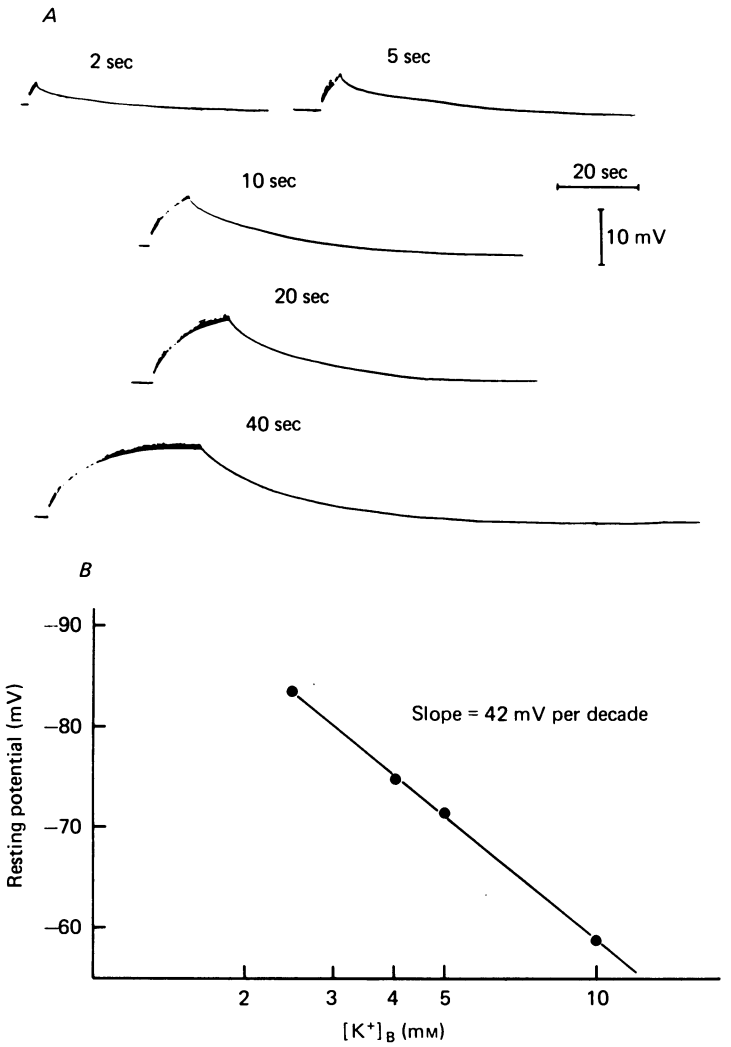

Fig. 1. *A*, pen writer traces showing the changes of the maximum diastolic potential during, and the resting potential after, stimulus trains of various lengths, at a stimulation frequency of 5 Hz. The full action potentials during the train are not well reproduced here. No action potentials were elicited after the trains. Train lengths given by each train are approximate. Bathing $[K^+] = 5$ mM. Resting potential $= -74$ mV. The sudden jump in the maximum diastolic potential, seen in these records at the onset of stimulation, occurs because the action potential duration after a period of quiescence is initially longer than the inter-stimulus interval used in this experiment (see later). Consequently, the second action potential is initiated from a less negative potential than the first action potential. *B*, typical dependence of the resting potential of guinea pig ventricular muscle (ordinate) on the K^+ concentration in the bulk solution perfusing the preparation (abscissa).

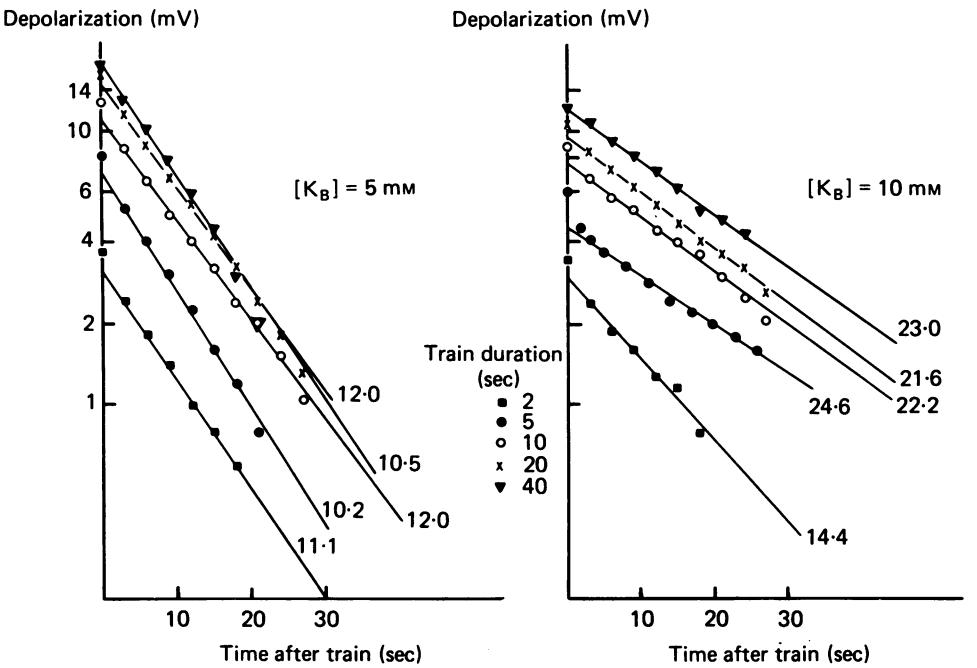

Fig. 2. Semilogarithmic plots of the recovery of the resting potential, after trains of different length (stimulation frequency 5 Hz). Recoveries are shown in solutions of two different bulk K concentrations (K_B), in the same preparation. The time constant of the recovery, given in seconds by each line, is larger in 10 mM-K_B than in 5 mM-K_B.

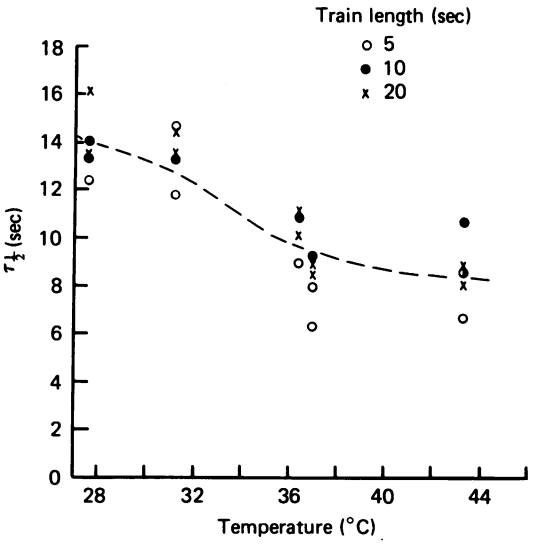

Fig. 3. Half-time of recovery of the resting potential after trains of different lengths, at temperatures between 27 and 44 °C. Stimulation frequency 5 Hz. Half-time is used, rather than time constant, for convenience. The Q_{10} of the recovery rate is about 1·5. K_B = 4 mM.

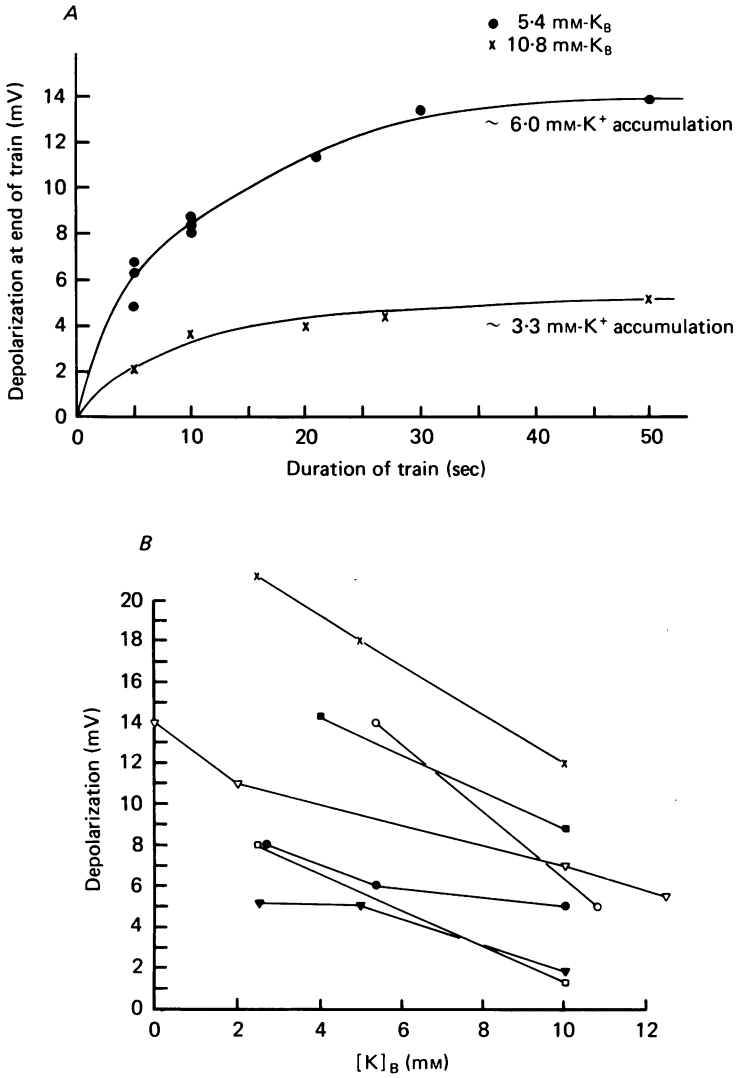

Fig. 4. *A*, depolarization of the resting potential after 5 Hz trains of various lengths (abscissa) in two different K_B solutions. The figures quoted for K^+ accumulation are derived by relating the resting potential depolarization to K_c changes, using the resting potential–K_B relationship of Fig. 1 *B*. *B*, maximum resting potential depolarization found after 5 Hz stimulation, as a function of K_B. Each line connects points measured in one preparation.

by cooling, and with strophanthidin (10^{-4} M). Fig. 3 shows that the half-time of the recovery is slightly smaller at higher temperatures. There is a lot of scatter in the results, but one can estimate the Q_{10} for the rate of recovery as 1·5. (For comparison, in frog heart Kline & Morad (1976) found a Q_{10} of 2·0 for the rate of decay of accumulated extracellular K^+). Strophanthidin was found to have no significant effect on the recovery rate. For example, in a preparation at 36·8 °C, with the bathing $[K^+] = 4$ mM, the half-time of the resting potential recovery following stimulation at 5 Hz was 3·8 sec (S.D. = 0·5 sec, $n = 6$) in the absence of strophanthidin, and 3·6 sec (S.D. = 1·1 sec, $n = 6$) with strophanthidin present. The relatively low Q_{10} of the recovery rate, and the absence of a large effect of strophanthidin, may suggest that most of the K^+ accumulated extracellularly is removed by diffusion to the bulk solution, rather than by pumping into the cells (but see Discussion).

The development of the depolarization

The time course of the development of K^+ accumulation cannot be studied by examining the time course of the maximum diastolic potential during a high frequency train of action potentials, since this is determined not only by the K^+ concentration in the extracellular clefts, but also by the known, rapidly changing gating variables. These may be responsible for a fast phase of repolarization seen sometimes in the first 1 sec after a train. Instead, therefore, we applied trains of different length and measured the depolarization produced just after each train (after the fast initial repolarization, if present). Specimen data for this protocol are shown in Fig. 1*A*.

The development of the depolarization obtained in this way (Fig. 4*A*) follows an approximately exponential time course. In Fig. 5*A* we plot the time constants of this development (τ_{acc} (train)), and of the subsequent recovery (τ_{acc} (recovery)), as a function of train frequency and length in one preparation. The data are rather scattered, but show little, if any, dependence on the length or frequency of the trains. This is in sharp distinction to the behaviour of the action potential shape described later.

The maximum depolarization of the resting potential is found to increase with the stimulation frequency (Fig. 5*B*). This increase presumably reflects the balance between at least two opposing factors: first, there are more action potentials per unit time, which tends to increase the K^+ accumulation, but secondly, the action potentials are shorter at higher frequency (see later) so that less K^+ probably enters the extra-cellular clefts per action potential.

Possible involvement of an electrogenic pump in the resting potential changes

In some preparations, when a high frequency train is applied, after an initial depolarization of the maximum diastolic potential there is a slow, partial, repolarization (Fig. 6). Furthermore, on termination of the train the resting potential sometimes goes transiently more negative than its steady-state value. This behaviour was seen in about 20 % of the preparations studied. Glitsch (1973) suggested that the partial repolarization during a train (in guinea-pig *atrium*) was due to an increase of the electrogenic Na/K pump current, caused by a rise in the intracellular Na concentration (Na_i). The rise in Na_i can stimulate the Na/K pump to reduce the

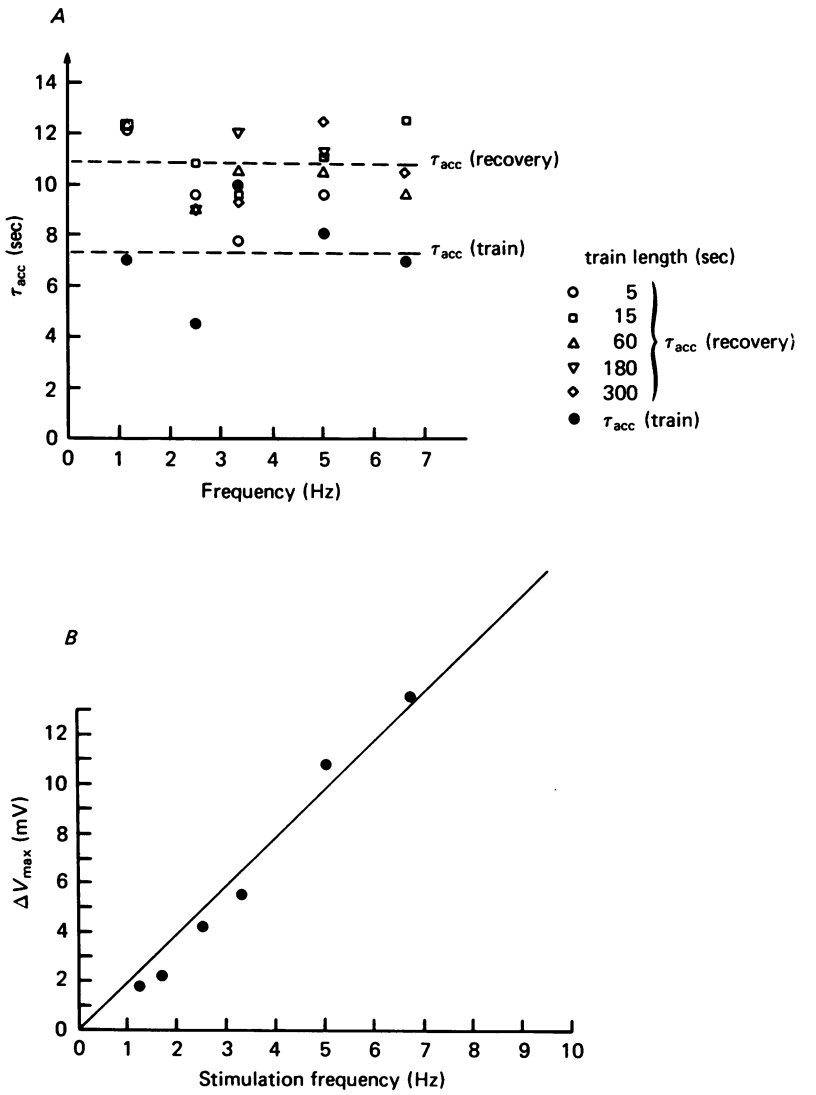

Fig. 5. *A*, time constants of the change in resting potential after a train (τ_{acc} (recovery)) and during a train (τ_{acc} (train)). τ_{acc} (train) is defined by the time course of the development of the depolarization measured just after the end of trains of different lengths (e.g. Fig. 4*A*). Thus, for each frequency of stimulation (abscissa), there is only one point for τ_{acc} (train) since trains of different lengths are needed to obtain this quantity, but values of τ_{acc} (recovery) are available from trains of different lengths at the same frequency. $K_B = 5$ mM. *B*, dependence of the maximum depolarization of the resting potential measured after a train (ordinate), found on stimulating at different frequencies (abscissa). $K_B = 5$ mM.

$[K^+]$ in the extracellular clefts (K_c) towards base line levels during long trains (Kunze, 1977), and also cause a K_c undershoot following cessation of activity (Kunze, 1977). This K_c undershoot, together with the extra outward current contributed by the pump (Glitsch, 1973; Gadsby & Cranefield, 1978), could account for the resting potential undershoot that we find. Occurrence of the undershoot did not seem to depend on train length in our experiments.

Effects of the bathing $[K^+]$ on the resting potential changes

After a high frequency train the resting potential recovers more slowly when the bathing $[K^+]$ (K_B) is higher (see Fig. 2). Factors determining the time course of the removal of accumulated extracellular K^+ are considered in the Discussion.

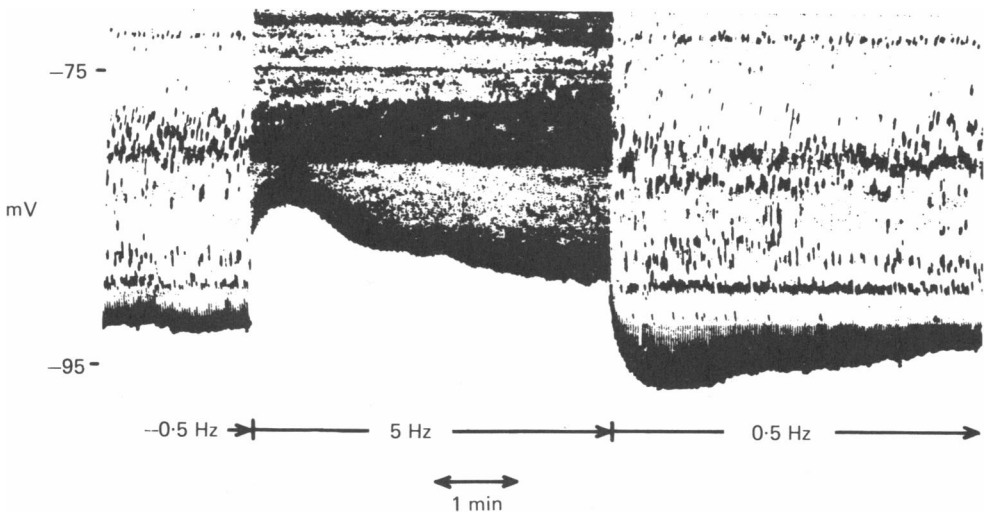

Fig. 6. After a period of 0·5 Hz stimulation, a 5 Hz train is applied (darker area, action potentials are upward deflections). The resting potential depolarizes, but then partially repolarizes during the train, and on returning to 0·5 Hz stimulation rate the resting potential goes transiently more negative than its control value (−92 mV). K_B = 2·7 mM.

Fig. 4 shows the development of the resting potential depolarizaton for a preparation in two different K_B solutions. Also shown are graphs of the maximum depolarization reached (after long trains), as a function of K_B, in seven different preparations (all at 5 Hz stimulation frequency). In general, a smaller depolarization is produced when K_B is higher. This may be partly because a given amount of K^+ entering the extracellular clefts produces a smaller fractional change of the cleft concentration when K_c is higher. However, it is also possible that there is less net K^+ entry into the clefts when K_c is higher. If we use the dependence of the resting potential on K_B in Fig. 1*B* to calculate the increase in extracellular $[K^+]$ from the depolarization of the resting potential shown in Fig. 4*A*, we find that the increase of K_c in 5·4 mM-K_B (~ 6·0 mM) is almost twice as large as that in 10·8 mM-K_B (~ 3·3 mM). Note, however, that it may be invalid to apply the steady state resting potential-K_B relation to the situation following a train, when electrogenic pumps may be more active (see above) and the distribution of K^+ in the extracellular clefts may be highly non-uniform.

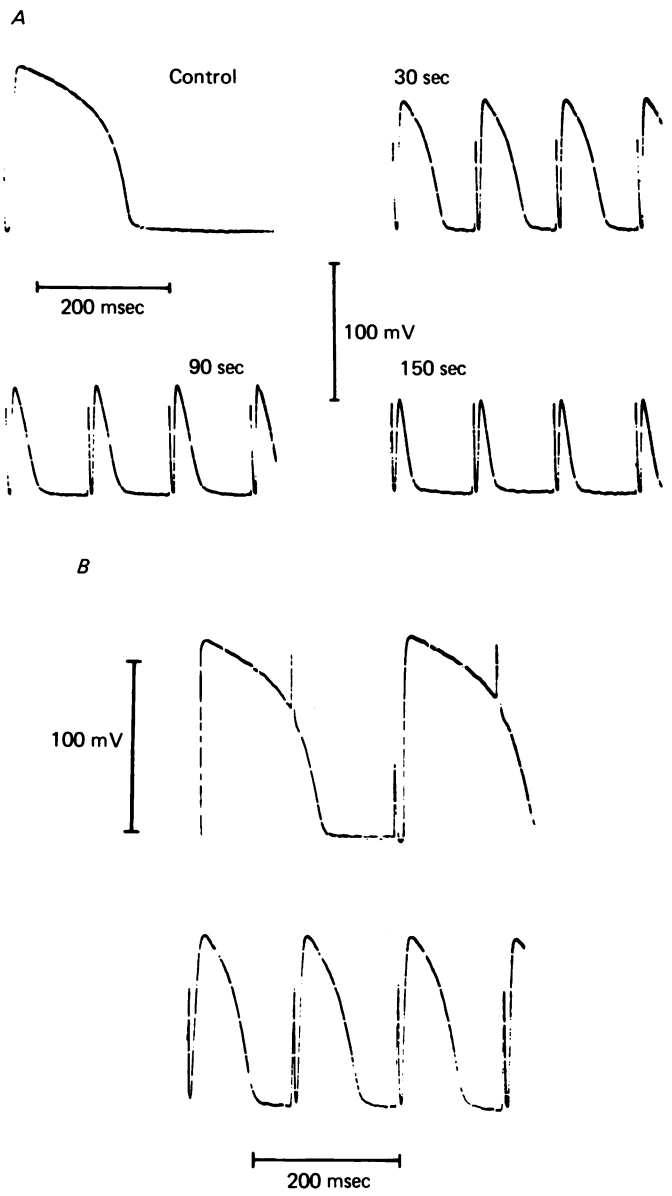

Fig. 7. *A*, shortening of the action potential during a train. The control action potential (at 0·5 Hz stimulation frequency) is shown, together with specimen action potentials at 30, 90 and 150 seconds after increasing the stimulation frequency to 5 Hz. The 200 msec time bar applies only to the control action potential. $K_B = 5$ mM. *B*, on suddenly increasing the stimulation frequency from 0·5 Hz to 6·7 Hz the action potential is initially too long for each stimulus to evoke an impulse (top). The apparent spike on the descending phase of the action potential is the stimulus artefact. Later (bottom) the action potential has shortened sufficiently for the ventricular cells to follow every driving stimulus. $K_B = 5$ mM.

Action potential changes during activity

During a train the action potential shortens (Fig. 7*A*), and takes up to 5 min to reach a steady, shortened shape. The action potential amplitude is reduced over a similar period. In Fig. 7*B* we can see that this shortening of the action potential is essential if high heart rates are to be achieved. Initially, the action potential duration, and therefore the refractory period, are long, and only every other stimulus elicits an action potential. Later, as the action potential shortens, action potentials are produced by every stimulus. This shortening is partly due to the time-dependent gated currents in the membrane (Beeler & Reuter, 1977). To eliminate effects due to these known, relatively short time constant, currents, we investigated the recovery of the action potential duration and amplitude after the end of a high frequency train by applying action potentials at a rate of one every two seconds after the train. In most preparations this rate is sufficiently low to produce little or no resting potential depolarization or shortening of the action potential. The time course of recovery of the action potential duration was the same whether it was studied using 0·5 or 0·1 Hz stimulation rate after the train. The action potential duration was measured at a potential 20 mV more positive than the maximum diastolic potential. The action potential amplitude was measured relative to the resting potential just before the action potential.

In Fig. 8*A* we see that the action potential duration and amplitude are reduced after a train, and recover slowly with time. It is well known that increasing the bathing K^+ concentration shortens the action potential (Weidmann, 1956; Hoffman, 1972). We investigated, therefore, whether the K^+ accumulation discussed earlier is responsible for the reduction of the action potential duration. In Fig. 8*B* we plot the normalized time course of recovery of the resting potential and action potential duration. Following a 5 sec train, the two recover with a similar time course. On increasing the train duration to 1 and 5 min, however, the time course of the action potential duration recovery becomes much slower, although the time course of the resting potential is unaffected. The time course of recovery of the resting potential is approximately exponential (Fig. 2), while the action potential duration recovers with a time course that is clearly not exponential (Fig. 8*B*, Fig. 12; Carmeliet, 1958). From this, it seems unlikely that there is any simple relationship between the action potential duration and resting potential, and thus no simple relationship between K^+ accumulation and the action potential duration (at least for long trains). For long trains, the recovery of the action potential amplitude is also much slower than that of the resting potential (Fig. 9), although usually not as slow as that of the action potential duration. A significant fraction of the amplitude recovery occurs when the resting potential is fully recovered, so only a portion of the reduction of the amplitude by a train is attributable to the resting potential depolarization.

It might be argued that, even if both the action potential duration and resting potential were only affected by K^+ accumulation, their recovery time courses could still be very different. Following the longer trains for example, removal of the last fraction of accumulation may have no significant effect on the resting potential, but may produce large changes in the action potential duration. The following experiments suggest that this is not so. After 2 min of stimulation at 5 Hz in 5·4 mM-K_B, the resting

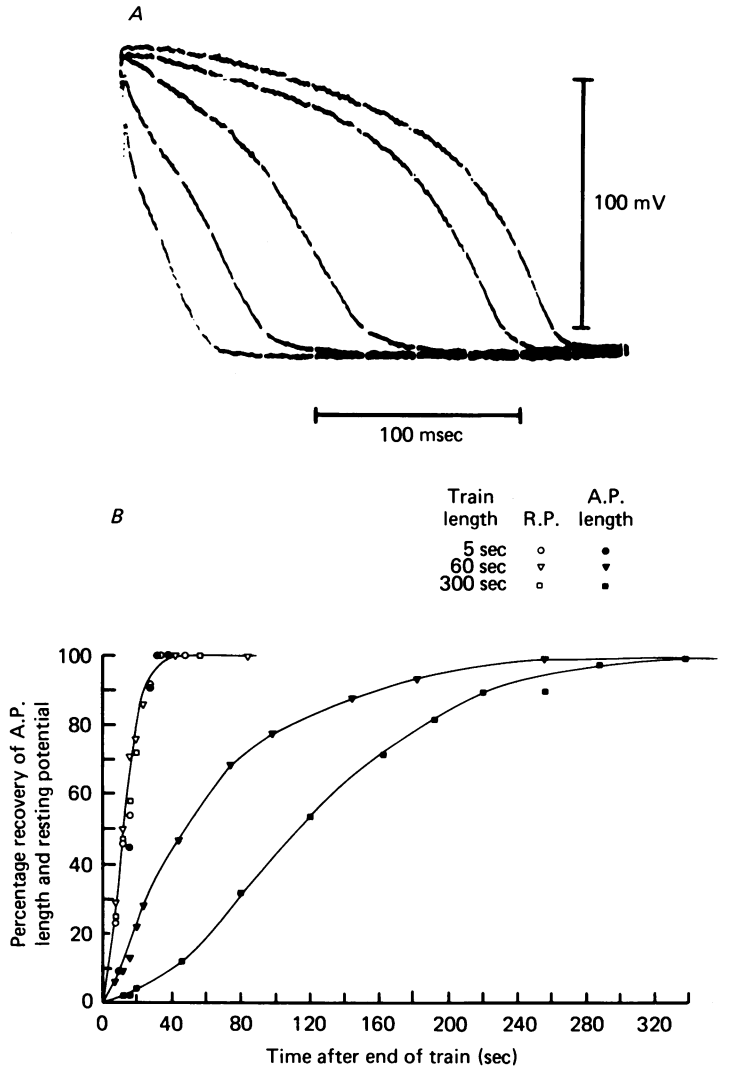

Fig. 8. *A*, after a 5 min train at 5 Hz frequency, the action potential is greatly shortened, and the amplitude reduced. The action potentials shown were recorded (subsequently, shortest action potential first) 30, 90, 150, 270, and 390 sec after the end of the train. The resting potential was at its control (pre-train) value throughout the period shown. K_B = 5 mM. *B*, normalized time course of recovery of the resting potential (R.P.) and the action potential (A.P.) length, after trains of various lengths at 5 Hz frequency. For long trains there is a clear dissociation of the resting potential and action potential duration time courses. K_B = 5 mM.

potential was depolarized by 8 mV and the action potential duration was almost halved. Doubling K_B to 10·8 mM produced a larger depolarization than that produced by the train, but the action potential (at 0·5 Hz stimulation rate) did not shorten nearly as much as during the train. This confirms the result of Reiter & Stickel (1968).

However, it is possible that the larger accumulation occurring deeper in the bundle of cells during a train (Kline & Morad, 1976) could produce a shorter action potential

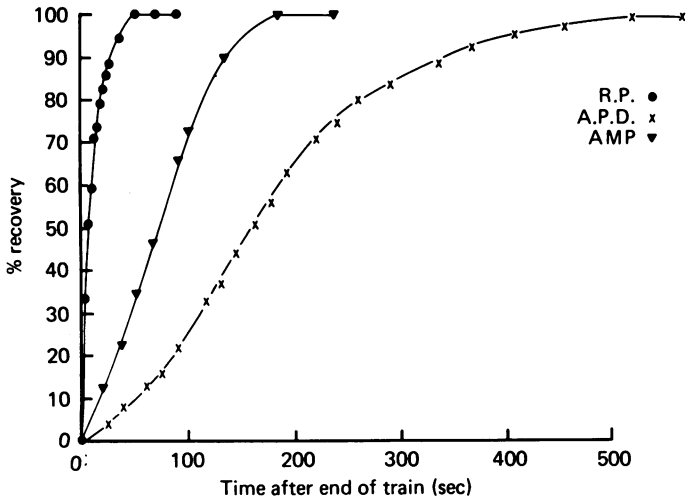

Fig. 9. Comparison of the (normalized) time course of recovery of the resting potential (R.P.), action potential duration (A.P.D.) and amplitude (AMP.) after a 5 min train at 5 Hz frequency. There is a clear dissociation of the time course of the three parameters using such long trains. $K_B = 5$ mM.

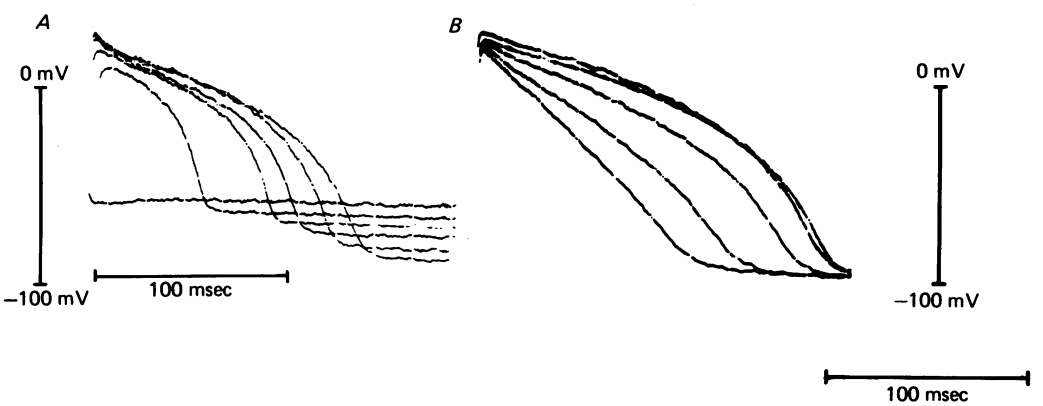

Fig. 10. *A*, action potentials recorded during a rapid change of K_B from 20 to 4 mM. In 20 mM-K_B the preparation was inexcitable. The traces shown were elicited 0, 30, 60, 90, 150 and 300 sec after the start of the K_B change. The 0 sec trace consists only of the stimulus artifact. Even inducing inhomogeneities of K_c in this way, we are unable to produce the amount of shortening that activity can result in, for a given depolarization of the resting potential. *B*, action potentials recorded during 0·5 Hz stimulation, at times 2, 4, 8 and 300 sec after a 2 min train at 5 Hz stimulation frequency, together with the pre-train action potential (the longest action potential near complete repolarization). At complete repolarization (not shown) the longest two action potentials had a maximum diastolic potential 8 mV more negative than the shortest action potential.

(at a given resting potential), than is produced by an increase in K_B. We attempted to test this hypothesis, by artificially imposing a K_c gradient through the preparation. The preparation was equilibrated in 20 mM-K_B, and then K_B was reduced to 4 mM. The K_B change was complete in less than 10 sec. However the resting potential took two minutes to reach its maximum hyperpolarization. This presumably reflects the

time for $[K^+]$ to equilibrate through the extracellular space. The estimated diffusion time across the extracellular space is $t \sim x^2/2D = 2$ min (with $x = 0{\cdot}5$ mm and $D = 10^{-5}$ cm^2/sec). (In view of this calculation it is surprising that the K_c equilibration after a train takes only 10 sec: this difference may reflect the two-dimensional structure of the preparation.) While the external $[K^+]$ is equilibrating, there will be a gradient of K_c, with the central part of the preparation initially having the value of K_c it had in 20 mM-K_B, while the external cells are surrounded by 4 mM-K_c. Fig. 10*A* shows action potentials recorded at various times during the $[K^+]$ change. Although the first action potential shown is much shorter than the final one, it is clear that its shape does not resemble that produced by a 5 Hz train in 5·4 mM-K_B (Fig.

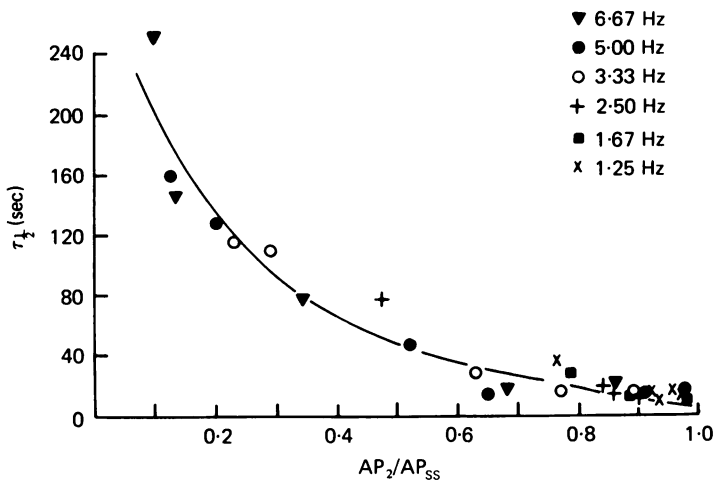

Fig. 11. Half-time of action potential duration recovery (ordinate) after trains of different frequencies, as a function of the fractional shortening of the second action potential after a train (abscissa). Within the scatter of the data, the recovery half-time depends only on the amount of shortening produced by a train and not on the frequency or length of the train which produced the shortening. $K_B = 5$ mM.

10*B*). Furthermore, for a given resting potential depolarization, even in the presence of a K_c gradient the shortening is much less than that produced by high frequency stimulation.

Similar results were found, when comparing the reduction of action potential amplitude produced by a train, with the effect of increasing K_B. (Indeed, in some preparations, a small increase of K_B from 5·4 mM can lead to an *increase* of the action potential amplitude.)

The dependence of the rate of recovery of the action potential duration on the amount of shortening

We have seen in Fig. 8 that action potential duration recovery is slower after longer trains. In general, the greater the amount of shortening produced, the larger is the half-time for the recovery of the action potential duration. In Fig. 11, the half-time is plotted against the ratio of the duration of the second action potential after a train (AP_2, elicited at 0·5 Hz stimulation rate) to the steady-state action potential duration reached after the train (AP_{ss}, at 0·5 Hz stimulation rate). The duration of the second

action potential after a train was used to define the shortening induced by the train, because the duration of the first action potential after the train may be influenced to a large extent by gating variables (such as those controlling I_x) which are changing rapidly just after termination of high frequency stimulation. Trains producing little shortening have a fast recovery of the action potential duration, whereas a greater initial shortening of the action potential is associated with a much slower recovery time course. No such phenomenon is seen in the kinetics of the resting potential recovery (Fig. 8*B*).

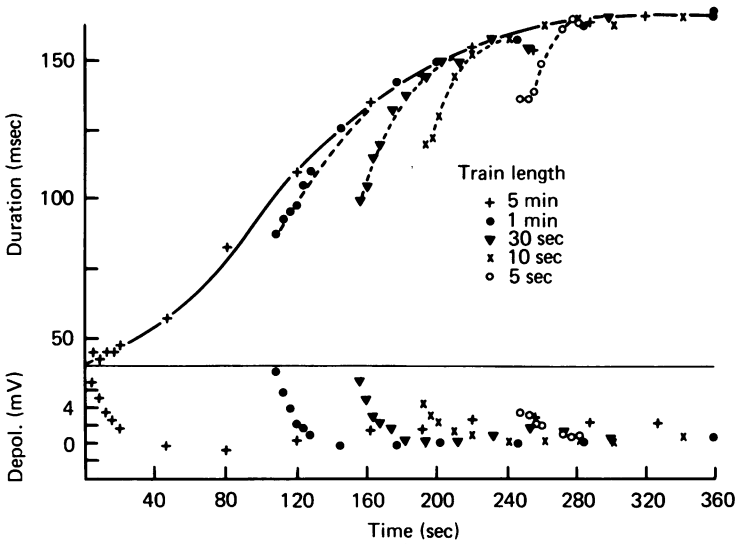

Fig. 12. Recoveries of action potential duration and resting potential after trains of different lengths, at 5 Hz frequency. The upper curves were shifted in time to make them overlap as much as possible. K_B = 5 mM. Although the curves overlap in the final phases of recovery, they do not in the early phases when, perhaps coincidentally, the resting potential is also changing. The recovery of the action potential is clearly non-exponential, in contrast to the exponential recovery of the resting potential (Fig. 2).

The half-time of recovery of Fig. 11 depends only on the fractional shortening produced by a train. In other words, it does not matter whether a certain amount of shortening is produced by a short, high frequency train or by a long, low frequency train. This might suggest that there is a single time-dependent factor controlling the action potential duration, but examining the whole time course of the action potential duration recovery after trains of different length apparently rules this out. The action potential duration recovery curves in Fig. 12 have been arbitrarily shifted along the time axis to superimpose as much as possible during the final phases. The final section of recovery from shorter trains superimposes on the recovery from the 5 min train, but there is a clear non-superimposition at earlier times. The period of non-superimposition coincides roughly with the period when the resting potential is depolarized. Thus, the initial section of the recovery of the action potential duration might be associated with the decay of K^+ accumulation or the resulting depolarization, whereas the later part of the recovery is probably due to some other factor. The slowing of the time course of recovery with longer trains might thus reflect the dominance of the other slower factor relative to K^+ accumulation.

We attempted to investigate the role of external K^+ in determining the rate of action potential duration recovery, by measuring the recovery half-times after 4 Hz trains up to 6 min long, in solutions with K_B between 2·5 and 10 mM. Over this range of K_B, there was no significant dependence of the action potential duration recovery rate on K_B (within the scatter of the data).

The effects of cardiotonic steroid intoxication

Cardiotonic steroids are known to shorten the action potential and depolarize the resting potential in most regions of the heart. The depolarization has been attributed to a blockage of the electrogenic pump current (Isenberg & Trautwein, 1974) and to the concomitant extracellular K^+ accumulation (Cohen *et al.* 1976*a*; Kunze, 1977). Since the action potential is shortened by an increase of the bathing K^+ concentration

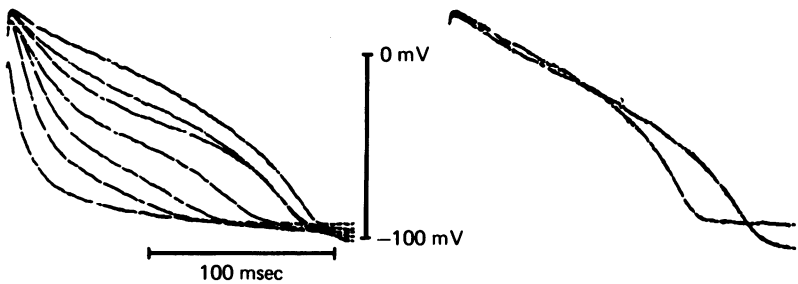

Fig. 13. Left-hand composite: action potentials recorded during the onset of cardiotonic steroid toxidity (strophanthidin, 10^{-4} M). Maximum depolarization of the resting potential was 13 mV. K_B = 4 mM. Right-hand composite: action potential shortening induced by raising K_B from 4 to 8 mM. For roughly the same depolarization as is found in strophanthidin, raising K_B produces much less reduction of the action potential duration.

(Weidmann, 1956; Hoffman, 1972), it is possible that cardiotonic steroids shorten the action potential as a result of the K^+ accumulation they produce (Cohen *et al.* 1976*a*; Hoffman, 1972; Miura & Rosen, 1978). If so, then we might be able to mimic the effects of toxic doses of cardiotonic steroids by raising K_B.

Fig. 13 shows action potentials recorded at various times after adding 10^{-4} M-strophanthidin to the bathing solution. The resting potential depolarized by 13 mV, and the action potential shortened until the preparation eventually became inexcitable. For comparison, Fig. 13 also shows the effects on the same cell of doubling K_B from 4 to 8 mM. A depolarization similar to that produced by the cardiotonic steroid is produced, yet the action potential shortens by a trivial amount. It appears, therefore, that K^+ accumulation alone cannot account for the shortening of the action potential produced by cardiotonic steroids.

Possible causes of the action potential changes, other than K^+ accumulation

Electrogenic pump current? It has been suggested (Glitsch, 1973) that the rise in Na_i produced by raising the stimulation frequency activates the electrogenic Na/K pump. The resulting outward current might be the cause of the reduced amplitude and duration of the action potential described earlier. In the presence of 10^{-4} M-strophanthidin, however, raising the stimulation frequency still shortens the action

potential. Furthermore, at the end of the high frequency train, there is almost no reversal of this shortening and the reversal which does occur is rapid and attributable to gating changes or removal of K^+ accumulation. This suggests that activity of the electrogenic pump is not necessary for the action potential shortening produced by an increase in stimulation frequency. However, this conclusion is only tentative since the strophanthidin itself shortens the action potential, and this shortening occurs more rapidly at a higher stimulation frequency (Moran, 1972).

Calcium-activated potassium conductance? Isenberg (1977) and Siegelbaum, Kass & Tsien (1977) have demonstrated the existence of a Ca^{2+}-activated K^+ conductance in Purkinje fibres, and there is circumstantial evidence for such a conductance in other cardiac preparations (Bassingthwaite *et al.* 1976). Such a mechanism could be invoked to explain the shortening produced by an increase of stimulation frequency and by cardiotonic steroid intoxication, since both of these are expected to raise the internal calcium concentration. This rise in Ca_i could shorten the action potential via an increased outward K^+ current in the plateau range of potentials. We attempted to investigate this possibility in two ways.

First, we applied a dye (3,3′-diethylthiadicarbocyanine, 10^{-4} M) which, in the erythrocyte, blocks the Ca^{2+}-activated K^+ conductance (even at much lower doses (Simons, 1976). We found a small (< 50 msec) lengthening of the action potential (at 0·5 Hz stimulation rate), and also that the recovery of the action potential duration after a train was somewhat faster. However, the majority of the shortening induced by a train, and the slow recovery afterwards, were still present. Furthermore, application of the dye did not prevent the action potential shortening induced by strophanthidin (10^{-4} M). If a Ca^{2+}-activated g_K is involved, therefore, it must differ from that of the red blood cell in its sensitivity to the dye.

Secondly, an increase of the external $[Ca^{2+}]$ from 2 to 10 mM was found to shorten the action potential (at 0·5 Hz stimulation rate), and also reduced the action potential duration reached after a train. Although this is consistent with an increased activation of the putative Ca^{2+}-activated g_K, other mechanisms could certainly be invoked to explain these results.

Human studies

The QT interval of the electrocardiogram (e.c.g.) provides a good measure of the human ventricular action potential duration (Olsson, 1972). It is well known that the QT interval is shorter in the steady state at higher heart rates. Trautwein, Kasselbaum, Nelson & Hecht (1962) have shown a similar frequency dependence of the action potential duration in isolated pieces of human ventricle, suggesting that hormonal or neural influences are not necessary to produce the frequency dependence of the QT interval.

In order to observe the effect of a controlled change in heart rate, we studied patients undergoing routine diagnostic or therapeutic pacing (see Methods). Fig. 14 shows the e.c.g. changes following a 3 min period of atrial pacing at 3 Hz. (No pacing was applied during the period of these graphs). The heart rate recovers very quickly, but the e.c.g. wave form, and in particular the QT interval, are still recovering up to 3 min after cessation of the pacing. It seems, then, that there are slow changes in the human ventricular action potential duration following a change in stimulation

frequency. The time course of these changes is similar to that seen in isolated pieces of guinea-pig ventricle.

It is possible that some of these slow changes are due to factors extrinsic to the heart, e.g. slow alterations in hormonal or neural control following the period of rapid stimulation. We cannot rule out such effects, but any major change in hormonal or neural factors might be expected to produce changes in the heart rate, while in Fig. 14 the rate recovers very quickly ($\sim$ 10 sec) compared to the time needed for recovery of the QT interval ($\sim$ 3 min). Of course it is possible that a combination of neural

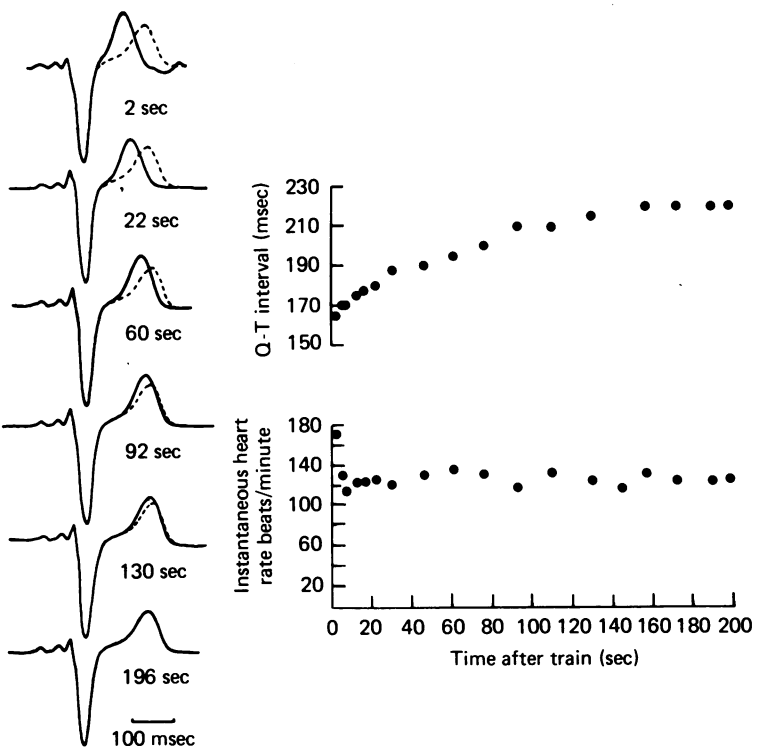

Fig. 14. Changes in human electrocardiogram on cessation of a 3 min period of diagnostic atrial pacing at a frequency of 3 Hz. Patient with Wolff–Parkinson–White syndrome. On the left are samples of the records (taken at various times after the pacing period) from which the graphs on the right were derived. Superimposed as a dotted line on each trace is the steady state (196 sec) record. The heart rate achieved a constant value of about 130/min in about 10 sec, whilst the QT interval took 3 min to reach a steady state.

and hormonal actions could cancel out in their effects on the heart rate after the first 10 sec following cessation of pacing, while still having a large effect on the ventricular action potential duration for the next 3 min, but this seems unlikely. Although these data were obtained from patients with heart problems (Wolff– Parkinson–White syndrome in the case of Fig. 14), the fact that the QT interval (in the steady state) is a function of heart rate in normal people strongly suggests that the slow changes seen here are not solely the product of diseased hearts.

DISCUSSION

Factors determining the time course of the resting potential changes

The time constants of the slowest changing currents in the ventricle are ~ 500 msec (Katzung & Morgenstern, 1977; see also McDonald & Trautwein, 1978). It seems unlikely, therefore, that the slow recovery of the resting potential (over ~ 10 sec) after a period of high frequency stimulation can be explained in terms of these gating variables. We attribute the depolarization of the maximum diastolic potential to K^+ accumulation (Kline & Morad, 1976; Kunze, 1977), although it is likely that gating changes play a role at early times, and stimulation of the electrogenic Na/K pump by a raised Na_i has an effect over longer periods.

The concentration of K^+ in the extracellular clefts is set by the balance of membrane K^+ currents, K^+ pumping into the cells, and K^+ diffusion to the bulk solution perfusing the preparation. For frog ventricle, Kline & Morad (1976) interpreted the Q_{10} of 2 that they found for the rate of decay of accumulated K^+ to mean that most of the excess K^+ was removed by pumping. The Q_{10} of 1·5 that we find for the recovery rate of the resting potential, and the lack of a large effect of strophanthidin, might seem to suggest that, in our preparation, diffusion to the bulk solution is the dominant means of removal of accumulated K^+. However, it is difficult to interpret the effects of a reduction of K^+ pump rate, because the resulting increase of K_c will alter the membrane K^+ currents and diffusion to the bulk solution (for further discussion, see Attwell *et al.* 1979*a*; Attwell *et al.* 1979*b*). In addition, changing the temperature will have a direct effect on the membrane K^+ fluxes. Thus, our data on the effects of temperature and strophanthidin are not conclusive proof that pumping is relatively unimportant. Conversely, the Q_{10} of 2 found by Kline & Morad does not prove that pumping is dominant in their preparation.

K^+ accumulation may be less important *in vivo*, where the heart is supplied by an intact capillary system (Franck & Langer, 1974), rather than perfused from the surface of a 1 mm thick preparation. Regions with a limited circulation, such as the border zone around an infarct, may be more similar to the condition of our preparations, and the present results on K^+ accumulation may be relevant to such pathological conditions.

Action potential changes: role of gating and K^+ accumulation

Part of the reduction of the action potential duration and amplitude, seen in the steady state after an increase of stimulation frequency, is the direct result of changes in Hodgkin–Huxley variables. At short inter-stimulus intervals, the factors responsible for repolarization of one action potential i.e. inactivation of the slow inward current (Reuter & Scholz, 1977) and activation of outward currents (Noble & Tsien, 1969; McDonald & Trautwein, 1978), will not have decayed completely before the next action potential. Hence, at high frequencies there will be a net increase of outward current which can shorten the action potential and reduce its amplitude. However, the recovery of the action potential duration and amplitude after a high frequency train is too slow (3–5 min) to be accounted for in terms of known gating variables.

K^+ accumulation plays some role in setting the action potential shape, although its effects are not always easy to dissociate from the effects of gating changes. Together they are probably responsible for the fast beat-to-beat changes in action potential shape seen when the stimulation frequency is suddenly changed. However, unlike the conclusion of Cohen *et al.* (1976*a*) for sheep Purkinje fibres, and Cohen *et al.* (1976*b*) for sheep ventricle, we consider the effects of K^+ accumulation in guinea pig ventricle to be relatively unimportant, except after short trains, for the following reasons. (i) The time course of recovery of the resting potential is much quicker than that of the action potential amplitude and duration (cf. Gibbs & Johnson, 1961). (ii) The half-time for recovery of the resting potential is independent of the action potential shortening produced by a train, whereas the action potential duration recovers more slowly after trains which produced greater action potential shortening. (iii) If, while stimulating at a low rate, the bathing $[K^+]$ (K_B) is raised in order to produce a depolarization similar to that produced by a high frequency train or by strophanthidin, the resulting shortening of the action potential is much less than that produced by the trains or strophanthidin (cf. Reiter & Stickel, 1968). This is so even if a gradient of extracellular $[K^+]$ is induced in the preparation (Fig. 10*A*).

There are two difficulties with the approach of point (iii) however. First, the dependence of the resting potential on the $[K^+]$ in the extracellular clefts (K_c) during low frequency stimulation need not be the same as that following a high frequency train if, for example, the electrogenic pump is stimulated by the train. Secondly, even if there is a unique relationship between the resting potential and K_c, the internal ion concentrations after a high frequency train (or with strophanthidin) are probably different to those with K_B raised, so the K_c dependence of the action potential shape may be different in the two cases.

Eisner *et al.* (1978) and Eisner & Lederer (1979) have found that inhibiting the Na/K pump by lowering K_B to zero produces changes of the action potential which are qualitatively similar to those produced by cardiotonic steroid intoxication, reinforcing our conclusion that the action potential shortening produced by cardiotonic steroids is not caused by an increase of K_c.

We conclude that there is a factor which provides intrinsic control of the action potential shape according to frequency, but which has no effect on the resting potential. Since the shortening actions of cardiotonic steroids occur more quickly at higher stimulation rates (Moran, 1972), an attractive hypothesis is that the same mechanism is responsible for the shortening induced by cardiotonic steroids and by an increase in stimulation rate.

Possible mechanisms for the control of the action potential shape

The number of possible mechanisms that can be postulated to explain our data is large, and we will simply give a brief discussion of those which we believe to be most plausible.

(1) *Gating changes.* Although no gating processes as slow as the action potential changes that we find have been reported in cardiac tissue, such slow gating has been reported in the Ranvier node (Neumcke, Fox, Drouin & Schwartz, 1976).

(2) *Extracellular ion concentration changes.* The diffusion time across the extracellular space of our preparation is about 2 min (see p. 452). In principle, therefore, the slow changes of the action potential could reflect the slow equilibration of an extracellular concentration. The most likely candidate for an ion having a significant

fractional change of concentration during activity (other than K^+) is Ca^{2+}, and Reiter & Stickel (1968) have proposed a role for such cleft $[Ca^{2+}]$ changes in controlling the action potential shape. However, the extracellular $[Ca^{2+}]$ is expected to decrease during a train of action potentials, whereas a decrease in the $[Ca^{2+}]$ bathing the ventricle is found to lengthen the action potential rather than shorten it.

(3) *Intracellular concentration changes.* Na_i and Ca_i may change significantly during activity, because of their influx with each action potential, and control the action potential shape via their effects on ionic currents or electrogenic pumps. Our experiments to test the role of a Ca^{2+}-activated K^+ conductance were inconclusive (p. 455), but we have tentatively ruled out the idea that stimulation of the Na/K pump by a raised Na_i is the sole mechanism responsible for the slow action potential changes (pp. 454–5).

Changes of the $[K^+]$ in the perfusing solution are expected to affect the removal of accumulated intracellular Na^+ and Ca^{2+}, via changes in the rate of the Na/K pump (which will affect Na^+ removal directly, and Ca^{2+} removal indirectly via Na/Ca exchange (Reuter & Seitz, 1968)), and via changes in the activity of any K^+-dependent Ca pump which may be present (Jones, Besch & Watanabe, 1977). Changing K_B from 2·5 to 10 mM has little effect on the recovery rate of the action potential shape, but conceivably an effect on the unknown factor controlling the shape is obscured by the effects of K_B on the membrane K^+ currents. Reducing K_B to a level where the Na/K pump is largely inhibited, gives a continuous reduction of the action potential amplitude and duration (as with cardiotonic steroids) without reaching a steady state from which the effects of a frequency change can be examined.

(4) *Intracellular messenger.* Changes in the heart rate might, via the membrane depolarization or changes in metabolic activity, alter the concentration of some intracellular chemical that controls membrane currents.

Human studies: implications of our results

Our results on the human e.c.g. suggest that there is a frequency-dependent control of the human ventricular action potential duration, which is apparently similar to that which we find in the isolated guinea pig ventricle. In other isolated ventricular preparations, catecholamines produce a small *lengthening* of the action potential (Morad & Rollett, 1972; Reuter, 1974), and acetylcholine has little or no effect on the action potential duration (Hoffman & Suckling, 1954). This suggests that, when the heart rate is raised by external hormonal or neural influences, the resulting shortening of the ventricular action potential may be due largely to the concomitant change of atrial rate, rather than a direct effect on the ventricle.

The intrinsic frequency-dependent control of the action potential shape, investigated in this study, may have several functionally important roles. First, the decrease in action potential duration produced by an increase of heart rate may be necessary to guarantee a sufficent diastolic interval for the fast and slow inward currents to recover from inactivation. This recovery from inactivation occurs more slowly at depolarized potentials (Gettes & Reuter, 1974), so the action potential shortening may be important in preventing heart block during periods of sustained tachycardia. Secondly, since the action potential duration adapts only slowly to a change of stimulation frequency, it will not easily follow rapid variations of driving frequency,

such as occur with an interpolated extrasystole, because the refractory period is too long (see Fig. 7*B*). It will, however, be able to follow gradual changes in the atrial driving rate.

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