Characteristics of Action Potentials of Hypertrophied Myocardium from Rats with Renal Hypertension

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SUMMARY We investigated the electrophysiological effects of cardiac hypertrophy induced by renal hypertension in rats by comparing transmembrane action potentials (AP) recorded from the papillary muscles of hypertensive (HBP) and normal (SHAM) rats. No significant difference was found between HBP and SHAM AP with regard to resting membrane potential (RMP), action potential amplitude (AMP), overshoot (OS), or maximum rate of rise of the upstroke. In contrast, the duration of 50% (APD50) and 75% (APD75) of repolarization to the RMP was significantly and consistently longer for HBP AP than for SHAM AP. The mechanism for prolonged HBP AP was investigated by changing extracellular fluid composition and by use of ion channel blockers. The responses of HBP and SHAM AP to various treatments differed in a quantitative rather than qualitative fashion. Exposure to Ca2+-containing or low-Na+-Tyrode’s solution produced a differentially greater decrease in APD50 and APD75 in HBP AP than in SHAM AP. Treatment with D600 also produced differential shortening of HBP AP, but its effect was limited to APD50. In contrast, exposure to Sr2+-containing and TEA-containing Tyrode’s solution produced an increase in APD50 and APD75, but the lengthening effect was not differentially greater in HBP than in SHAM AP. Treatment with Ca2+-free Tyrode’s solution had little effect on APD in either HBP and SHAM rats. None of the treatments had a significant differential action on RMP or AMP in HBP AP as compared to SHAM AP. Our results show that AP prolongation is a specific and consistent feature of hypertrophied myocardium and that the changes responsible for prolonged HBP AP are quantitative rather than qualitative in nature. The specific differential effects of high Ca2+ concentration ([Ca2+]o) and low Na+ concentration ([Na+]o) on the duration of HBP AP indicate that the membrane sensitivity to these ions is altered in hypertrophied myocardium and that one possible explanation for prolonged HBP AP is slowed inactivation of a Ca2+-inactivated inward current. Circ Res 47: 443-454, 1980

CARDIAC hypertrophy is known to be associated with electrophysiographic, ultrastructural, and contractile alterations, but only a few studies on the cellular electrophysiology of hypertrophied myocardium are available. Those studies have reported a variety of action potential changes but have disagreed on the nature of the alterations and on when, during the course of hypertrophy, they occurred.

For example, Uhley (1961) studied the electrical effects of hypertrophy in rats made hypertensive for 5-8 months. He reported no apparent differences in transmembrane action potentials recorded from various epicardial sites of normal and hypertrophied hearts. This study, however, was limited to the only action potential parameter measured was total amplitude.

Most other studies of the electrophysiological effects of cardiac hypertrophy have used an entirely different model in which right ventricular hypertrophy (RVH) is induced in cats by acutely banding the pulmonary artery (Spann et al., 1967). Kaufmann et al. (1971) used this model and found no significant difference between action potentials from normal and hypertrophied muscles when resting potential, upstroke velocity, overshoot, and action potential duration were compared. In contrast, Bassett and Gelband (1973) found a depression of the plateau phase of the action potential 3 days after pulmonary artery constriction. This plateau depression, which was not associated with any other significant changes in the action potential, was less marked after 7-10 days, and the action potentials became normal again at 21 and >90 days. The papillary muscles studied were obtained from cats that were considered to have hypertrophy uncomplicated by congestive heart failure (CHF). In a subsequent study of action potentials recorded from right ventricular papillary muscles obtained from cats that developed evidence of CHF, Gelband and Bassett (1973) reported marked action potential alterations. Action potentials of hypertrophied muscles from cats with CHF showed prolonged durations and decreases in resting potential, overshoot, and maximum upstroke velocity.

In another study, Tritthart et al. (1975) subjected cats to pulmonary artery constriction for 3-6 weeks.
Of 12 cats studied, six were considered to have "mild" hypertrophy and six were considered to have "severe" hypertrophy. Compared to control action potentials, those recorded from the "mild" group had increased resting potentials, no change in overshoot, a decrease in upstroke velocity and conduction velocity, and a modest prolongation of duration. Action potentials recorded from the "severe" group showed an increase in overshoot, a more pronounced decrease in upstroke velocity, an increase in conduction velocity, and a somewhat more prolongation of action potential duration.

In the chronic phase (5–6 months) of RVH, Ten Eick et al. (1977, 1978) reported that action potentials from hypertrophied myocardium were 30–150 msec longer than those of controls. The lengthening of action potentials in RVH preparations was attributed to slower activation of outward K+ current and the development of a negative slope region in the current-voltage relation of the background current.

It has also been reported that spontaneously hypertensive rats (SHR) (Hayashi and Shibata, 1974; Heller et al., 1978; Heller and Stauffer, 1979) and rats made hypertensive with deoxycorticosterone acetate (DOCA) (Heller and Stauffer, 1979) have longer action potentials than do controls. Action potentials recorded from cardiac tissue taken from the heart of a 42-year-old female with hypertrophic cardiomyopathy have been reported to be longer than control action potentials recorded from papillary muscles taken from patients undergoing mitral valve replacement (Coltart and Jesselsohn, 1978, 1979; Strobeck et al., 1980).

The variability in results from studies on the electrophysiological effects of cardiac hypertrophy may be related, at least in part, to the models used to produce hypertrophy. For example, in the RVH model, the acute pressure overload imposed may produce uncertain and variable degrees of damage to a chamber that normally functions against low pressures. In the SHR model, the nature of the hypertrophy and the relation between elevated blood pressure and hypertrophy are uncertain (Cuttilletta et al., 1978).

The present study therefore was undertaken to investigate the electrophysiological effects of cardiac hypertrophy induced by a model that imposes a more gradual pressure overload on the heart. A useful model for producing this kind of pressure overload is renal hypertension in the rat. Advantages of this model include the availability of ultrastructural (Loud et al., 1978; Wendt-Gallitella et al., 1979) and mechanical (Jacob et al., 1977; Strobeck et al., 1980) data for correlation with electrical alterations as well as the close resemblance of hypertension in the rat to hypertensive disease in humans. We have found that action potentials recorded from hypertrophied myocardium produced by this model are consistently and substantially prolonged. Gulch et al. (1979), in a recent study using the same model, has reported very similar results with regard to action potential duration. Preliminary accounts of some of the results in this paper have been reported previously (Aronson and Jesselsohn, 1978, 1979; Strobeck et al., 1980).

Methods

Experimental Procedures

Male Wistar rats weighing 160–180 g were made hypertensive by a procedure similar to that described by Wolinsky (1971). This procedure involves placing a silver clip (0.22-mm aperture) around the left renal artery of experimental rats. Age-matched animals were subjected to a sham operation identical to that used in experimental rats except that no clip was placed on the left renal artery. The animals were maintained on water and standard rat chow (Tekland) given ad libitum.

Postoperatively, systolic blood pressure was measured in both clipped and sham-operated animals at weekly intervals for the first 4 weeks and then every 10–14 days. Hypertensive rats did not develop any abnormalities in serum electrolytes, blood urea nitrogen, or creatinine. Rats were lightly anesthetized with ether, and systolic blood pressure measurements were obtained at room temperature by the tail cuff microphonic manometer method. They were considered hypertensive when the systolic blood pressure was ≥170 mm Hg.

All solutions were made with glass-redistilled water and gassed with 95% O2, 5% CO2. Normal Tyrode's solution had a pH of 7.2 and the following composition in mM/liter: Na+, 154.2; Ca2+, 2.4; Mg2+, 0.5; K+, 4.0; Cl−, 147.3; H2PO4−, 1.8; HCO3−, 12.0; glucose, 5.5. In experiments in which the ionic composition of the solution was altered by more than 2 mM, the total osmolarity was maintained constant by a compensatory increase or decrease in the concentration of NaCl or by the addition of sucrose.

For Tyrode's solution in which external Na+ concentration ([Na+]o) was reduced, sucrose or choline chloride (Eastman Kodak) was used to replace NaCl. Atropine sulfate (Sigma Chemical Co.; 1.5 × 10−5 M) was added to choline chloride-substituted Tyrode's solution. Tetraethylammonium (TEA) chloride (Eastman Kodak) and D-600 hydrochloride (Knoll, A.G.) were added to Tyrode's solution from freshly prepared stock solutions.

Rats were used for experiments 7–14 weeks postoperatively. These animals were killed inagematched pairs so that one hypertensive and one normal animal was used in each experiment. Animals were anesthetized with ether, the heart was excised, and papillary muscles were removed from the left ventricles of hypertensive and normotensive sham-operated animals. The papillary muscles were then mounted in a tissue bath perfused with Tyrode's solution maintained at 34–35°C. The preparations were driven regularly at a cycle length of 1000 msec and allowed to equilibrate in the tissue.
bath for an hour before the first recordings were taken. Action potentials were recorded simultaneously from hypertrophied and normal papillary muscles.

Glass microelectrodes filled with 3 M KCl were used to obtain electrical recordings. External stimuli were delivered to the tissue through bipolar Teflon-coated silver wires. The timing of the stimulating pulses was regulated by a digital timing system interfaced with a pulse generator connected to a stimulus isolation unit. The maximum rate of rise of the action potential upstroke was obtained by electronic differentiation of the upstroke; the differentiating circuit had a linear output from 0-500 V/sec and was used with appropriate input capacity neutralization.

Photographic records were obtained with a Tektronix C5A Polaroid oscilloscopic camera.

Whereas renal hypertension in the rat is a dependable model for producing cardiac hypertrophy by pressure overload, the rat myocardium is known to differ from that of other mammalian species with respect to some electrophysiological and mechanical characteristics. For example, the rat ventricular action potential lacks a plateau phase and shows a rather slow phase of terminal repolarization (Hoffman and Cranefield, 1960). Rat myocardium also tends to show a negative staircase (Langer et al., 1975). These differences from other mammalian species do not necessarily vitiate the usefulness of investigating the effects of cardiac hypertrophy in the rat myocardium.

We have used ion substitutions and so-called ion channel-blocking agents to investigate the mechanisms possibly underlying the prolonged action potentials of hypertrophied myocardium. On one hand, experiments in which ionic composition is altered cannot give unequivocal results because the alterations in the action potential that result always will have more than one possible explanation. For example, later we will give evidence that increasing external [Ca$^{2+}$], produces shortening of the action potential. This result could be caused by an increased outward current induced by raised Ca$^{2+}$, by a decrease in an inward current inactivated by Ca$^{2+}$ or by a combination of such effects. Furthermore, under steady state conditions, changes in extracellular ion concentration also will influence intracellular ionic composition which could, in turn, account for changes in the action potential.

On the other hand, ion substitution experiments performed in a comparative fashion, as reported in this study, place certain constraints on the number of possible mechanisms and may expose differences in membrane properties between normal and hypertrophied myocardium. For example, our finding that increased [Ca$^{2+}$], shortens action potentials of hypertrophied myocardium to an extent substantially greater than that of normal action potentials suggests that an increased current carried by Ca$^{2+}$ is not responsible for the prolonged action potentials associated with hypertrophy.

The conclusions drawn from experiments in which blocking agents (D600, TEA) were used depend on the specificity of action of these compounds. This problem is discussed further with respect to D600 under Results. To our knowledge, there is no evidence to suggest that TEA has any significant effect other than blocking outward current in cardiac muscle (Kenyon and Gibbons, 1979). This, of course, does not exclude the possibility that TEA may have other as yet undefined actions.

The object of our experiments was to investigate possible mechanisms by detecting differences in the response to various treatments of action potentials of normal and hypertrophied myocardium. The presence of an appropriately documented differential response of hypertrophied myocardium to a particular treatment (see Data Analysis, below) was considered to indicate the presence of a change in electrical behavior associated with hypertrophy. This comparative approach differs from attempting to define absolute ionic mechanisms by the techniques employed in this study.

Data Analysis

The following action potential (AP) parameters were measured from photographic records obtained from hypertrophied papillary muscles removed from hypertensive (HBP) rats and normal papillary muscles from sham-operated (SHAM) rats driven at a cycle length of 1000 msec: diastolic resting membrane potential (RMP), action potential amplitude (AMP), overshoot magnitude (OS), action potential duration to 50% (APD$_{50}$) and 75% (APD$_{75}$) of complete repolarization to the RMP, and maximum rate of rise of the upstroke [(dV/dt)$_{max}$]. The RMP was assessed by withdrawing the microelectrode from the cell at the end of each experiment.

AP analyzed were obtained from maintained, random, single impalements in single cells from each HBP and SHAM muscle. All AP recorded under control conditions from 49 HBP and 53 SHAM animals were analyzed (see Table 2). Based on this data, in subsequent experiments using ion substitution or blocking agents, fibers with an RMP or AMP more than a standard deviation below the population means under control conditions were considered unhealthy; the results from such experiments were not accepted if either the HBP or SHAM muscle had a low value for either of these parameters under control conditions.

Parameters used to characterize HBP and SHAM animal groups were systolic blood pressure (BP), body weight (BW), and dry heart weight (HW).

The relationship between group characteristics and AP parameters, as well as interrelationships between the various AP parameters themselves, was assessed by determining the correlation coefficients ($r$) for these variables. Statistical significance of differences in the char-
characteristics of animal groups and AP (HBP SHAM) was assessed by applying analysis of variance which employs the variance ratio, $F$, to determine significance. A sufficiently high $F$ value indicates that a statistically significant difference exists between the group means of the variables under consideration. Statistical significance of differences in AP parameters of HBP and SHAM rats was assessed by applying the multivariate extension of the univariate $F$ test. Only the measured AP parameters described above were used in the multivariate analysis.

For experiments in which extracellular fluid was altered or drugs were given, the results of the effects of these treatments on AP parameters were also assessed by analysis of variance. $F$ values were determined for group differences, treatment differences, and interaction. Significant group differences are those that exist between HBP and SHAM AP parameters that are independent of the treatment given. Significant treatment differences are those that occur in AP parameters as the result of treatment, and that are independent of whether the animal belongs to the HBP or SHAM group. Interaction refers to a significant differential action of the treatment on the HBP as compared to the SHAM AP parameter under consideration.

Each AP analyzed was obtained from a maintained single impalement in a single cell of each muscle. Therefore, in the tables that follow, the number of animals given in parentheses is equal to the number of impalements, since there was one impalement per muscle.

**Results**

**Characteristics of Normal and HBP Animals**

Rats subjected to clipping of the left renal artery developed hypertension within 10–14 days after operation. In most HBP animals, the elevated BP persisted for the duration of the postoperative period until the rats were used for an experiment. The HBP animals rarely showed evidence of heart failure, and their left ventricles were visibly thicker than normal.

Rats subjected to a sham-operation did not develop hypertension, and the BP of these animals remained constant at much lower levels.

Table 1 summarizes and compares the characteristics of the HBP and SHAM animal groups. Both BP and HW were significantly greater in HBP rats, but BW did not differ significantly between animal groups. A significant correlation was found between HW and BW in both HBP ($r = 0.53; P < 0.01$) and SHAM ($r = 0.44; P < 0.01$) animals. SHAM animals also showed a significant correlation between BW and number of postoperative weeks ($r = 0.62; P < 0.01$), but the correlation between these variables was not significant in HBP animals ($r = 0.04; P > 0.50$).

**Table 1 Characteristics of Animal Groups**

<table>
<thead>
<tr>
<th></th>
<th>BP (mm Hg)</th>
<th>HW (g)</th>
<th>BW (g)</th>
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<tbody>
<tr>
<td>HBP</td>
<td>210 ± 22 (48)</td>
<td>1.36 ± 0.13 (47)</td>
<td>391.9 ± 47.7 (48)</td>
</tr>
<tr>
<td>SHAM</td>
<td>127 ± 23 (49)</td>
<td>0.99 ± 0.14 (50)</td>
<td>427.6 ± 49.2 (51)</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD. Numbers in parentheses represent number of animals.

* $P < 0.01$, HBP vs. SHAM.

**AP Changes Associated with Hypertrophy**

Figure 1 shows representative AP recorded simultaneously from three different pairs of HBP and SHAM papillary muscles driven regularly at 1 Hz. In each case, the HBP AP (Fig. 1A–C) is substantially longer than the SHAM response (Fig. 1, D–F). Figure 1 also shows that, under the same conditions, the prolonged course of repolarization in HBP AP may show much wider variation in shape than the course of normal repolarization in SHAM AP.

Various parameters of HBP and SHAM AP are summarized and compared in Table 2. As suggested by the records in Figure 1, the most dramatic difference between HBP and SHAM AP is in duration. Both APD$_{50}$ and APD$_{75}$ were consistently and quite significantly prolonged in HBP AP. The RMP, AMP, and OS did not differ significantly in HBP AP as compared to SHAM AP. No statistically
significant correlation was found between either APD50 and APD75 and any of the other AP parameters listed in Table 2. A significant correlation was found between APD50 and APD75 in both HBP (r = 0.97; P < 0.01) and SHAM (r = 0.88; P < 0.01) AP. Neither HBP nor SHAM data showed a statistically significant correlation between any of the AP parameters in Table 2 and BP, HW, BW, or number of postoperative weeks. No significant difference was found in (dv/dt)max of HBP (160 ± 35 V/sec; n = 11) AP as compared to SHAM (154 ± 34 V/sec; n = 12) AP.

**Effects of Altering Extracellular [Ca2+]o**

We studied the effects of raising [Ca2+]o to 12 mM (5 times normal) in 13 preparations (seven HBP, six SHAM). The results of these experiments are summarized in Table 3 and show: (1) that there were significant group differences in APD50 and APD75 but not in RMP or AMP, (2) that treatment with high [Ca2+]o produces highly significant shortening of APD50 and APD75 in both HBP and SHAM AP but has no significant effect on RMP or AMP, and (3) that the extent of shortening induced by high [Ca2+]o is significantly greater in HBP AP than in SHAM AP, but high [Ca2+]o has no differential action on either RMP or AMP.

The data from these experiments are summarized in Table 4 and show: (1) that there were significant group differences in APD50 and APD75 but not in RMP or AMP, (2) that there were significant reductions in RMP and AMP during treatment with Ca2+-free Tyrode's in both HBP and SHAM AP but no significant effect on APD50 or APD75 in either group, and (3) that there was a significant differential effect on APD50 owing to a lengthening action on HBP AP and a shortening action on SHAM AP. However, calcium-free Tyrode's did not prove to have a significant differential effect on APD75, RMP, or AMP.

Figure 3 shows traces from one experiment in which preparations were exposed to calcium-free superfusate. This experiment also shows that the effects of [Ca2+]o are not shared by [Mg2+]o. Panels A and D show that exposure to calcium-free solution has qualitatively similar effects on both HBP and SHAM AP. Perfusion with Ca2+-free Tyrode's solution produces a reduction in OS and alters the course of the earlier phases of repolarization most strongly. Panels B and E show that, during exposure to Tyrode's solution containing 7.2 mM Ca2+ and no Mg2+, repolarization is hastened and the OS is restored. Panels C and F show that the effects of exposure to Ca2+-free Tyrode's solution with 7.2 mM Mg2+ more closely resemble those seen in calcium-free Tyrode's (Fig. 3A and D) than in Tyrode's containing 7.2 mM Ca2+ (Fig. 3B and E). Similar differential actions of [Mg2+]o and [Ca2+]o on repolarization were obtained in an additional experiment.

**Effects of Extracellular [Sr2+]o**

In addition, to determine whether the actions of high [Ca2+]o on repolarization are relatively specific,

<table>
<thead>
<tr>
<th>Table 2</th>
<th>AP Parameters for Animal Groups</th>
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<tbody>
<tr>
<td></td>
<td>RMP (-mV)</td>
</tr>
<tr>
<td>HBP</td>
<td>78 ± 6.5 (49)</td>
</tr>
<tr>
<td>SHAM</td>
<td>81.5 ± 6.1 (53)</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± sd. Numbers in parentheses represent number of preparations.

*P<0.01, HBP vs. SHAM.

Data in the upper part of the table are presented as mean ± SD. In the lower part of the table, group refers to the differences between HBP and SHAM preparations independent of treatment; treatment refers to differences produced by the treatment (12 mM [Ca2+]o) independent of whether the preparation was from an HBP or SHAM animal; interaction refers to a differentially greater treatment effect on either the HBP or SHAM preparation. F = F value and F = probability value for HBP vs. SHAM. Degrees of freedom for F test = 1 and 11. In this and all subsequent tables, the mean values are from maintained impalements from single cells in each muscle.
we studied the effects of $[\text{Sr}^{2+}]_0$ in 12 preparations (six HBP, six SHAM). In these experiments, Ca$^{2+}$-free Tyrode’s solution containing 12 mM Sr$^{2+}$ was used. The results are summarized in Table 5 and show: (1) that significant group differences were detected for APD$_{50}$, APD$_{75}$, and RMP but not for AMP; (2) that treatment with $[\text{Sr}^{2+}]_0$ had a highly significant lengthening effect on APD$_{50}$ and APD$_{75}$ and produced a significant loss of RMP in both HBP and SHAM AP but had no significant effect on AMP; and (3) that despite the highly significant effects of $[\text{Sr}^{2+}]_0$, no statistically significant differential action in the extent of these effects on APD$_{50}$, APD$_{75}$, or RMP could be demonstrated for either animal group.

The records in Figure 4 are from one of the experiments with 12 mM Sr$^{2+}$. Panels A and D show that perfusion with Ca$^{2+}$-free Tyrode’s solution containing 12 mM Sr$^{2+}$ increased duration and decreased RMP in both HBP and SHAM AP. The alterations induced by treatment with Sr$^{2+}$-containing, Ca$^{2+}$-free Tyrode’s solution are qualitatively similar in both HBP and SHAM AP. Panels B and E show that the effects of $[\text{Sr}^{2+}]_0$ can be reversed largely by perfusion with Sr$^{2+}$-containing Tyrode’s solution containing 12 mM Sr$^{2+}$. The records in Figure 5 are from one of the experiments with 12 mM Sr$^{2+}$. Panels A and D show that perfusion with Ca$^{2+}$-free Tyrode’s solution containing 12 mM Sr$^{2+}$ increased duration and decreased RMP in both HBP and SHAM AP. The alterations induced by treatment with Sr$^{2+}$-containing, Ca$^{2+}$-free Tyrode’s solution are qualitatively similar in both HBP and SHAM AP. Panels B and E show that the effects of $[\text{Sr}^{2+}]_0$ can be reversed largely by perfusion with Sr$^{2+}$-containing Tyrode’s solution containing 12 mM Sr$^{2+}$.
The effects of D600 were considered to be of interest because this drug is presumed to block the slow ion channel thought to carry Ca\(^{2+}\) and Na\(^+\). Unfortunately, D600 appears to influence not only the magnitude of the slow inward current but also its kinetics (Nawrath et al., 1977) and the magnitude of certain outward currents (Kass and Tsien, 1975; Nawrath et al., 1977). Nevertheless, because [Ca\(^{2+}\)], strongly influences the course of repolarization, we studied the effects of D600 in 10 preparations (six HBP, four SHAM). The results of these experiments are summarized in Table 6 and show: (1) that there are significant group differences in APD\(_{50}\) and APD\(_{75}\) but not in either RMP or AMP, (2) that treatment with D600 produces highly significant decreases in APD\(_{50}\) and APD\(_{75}\) in both HBP and SHAM AP but has no significant effect on RMP or AMP in either group; (3) that the extent of the shortening action of treatment with D600 on APD\(_{50}\) but not on APD\(_{75}\) was significantly greater in HBP AP than in SHAM AP; and (4) that the relative extent of change in RMP under D600 treatment is greater for SHAM AP than for HBP AP, even though the effect of the drug on RMP is not significant for either group.

Effects of Decreasing Extracellular [Na\(^+\)].

The effects of lowering [Na\(^+\)]\(_o\) progressively in an containing normal (2.4 mM) [Ca\(^{2+}\)]\(_o\). Panels C and F show that exposure to Ca\(^{2+}\)-containing (12 mM) Tyrode's solution without Sr\(^{2+}\) produces still further shortening of both HBP and SHAM AP. The shortening effect of high [Ca\(^{2+}\)], on the HBP AP is quite marked and, in this situation, the HBP and SHAM AP resemble each other rather closely.

### Table 5 Effects of 12 mM [Sr\(^{2+}\)]\(_o\) on HBP and SHAM AP Parameters

<table>
<thead>
<tr>
<th></th>
<th>APD(_{50}) (msec)</th>
<th>APD(_{75}) (msec)</th>
<th>RMP (mV)</th>
<th>AMP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBP</td>
<td>SHAM</td>
<td>HBP</td>
<td>SHAM</td>
</tr>
<tr>
<td>Control</td>
<td>41.5 ± 20.4</td>
<td>73.2 ± 18.0</td>
<td>77.0 ± 3.4</td>
<td>106.7 ± 8.3</td>
</tr>
<tr>
<td>12 mM [Sr(^{2+})](_o)</td>
<td>73.2 ± 18.0</td>
<td>125.8 ± 42.7</td>
<td>66.5 ± 6.3</td>
<td>101.1 ± 7.7</td>
</tr>
<tr>
<td></td>
<td>15.79</td>
<td>23.19</td>
<td>4.15</td>
<td>0.069</td>
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<td></td>
<td>0.003</td>
<td>0.013</td>
<td>0.021</td>
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</tr>
<tr>
<td></td>
<td>7.54</td>
<td>12.80</td>
<td>4.86</td>
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<td>0.58</td>
<td>0.43</td>
<td>1.07</td>
<td>0.40</td>
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</table>

Data in the upper part of the table are presented as mean ± SD. In the lower part of the table, group refers to the differences between HBP and SHAM preparations independent of treatment; treatment refers to differences produced by the treatment (12 mM [Sr\(^{2+}\)]\(_o\)) independent of whether the preparation was from an HBP or SHAM animal; interaction refers to a differentially greater treatment effect on either the HBP or SHAM preparation. F = F value and P = probability value for HBP vs. SHAM. Degrees of freedom for F test = 1 and 10.
TABLE 6 Effects of D600 on HBP and SHAM AP Parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>APD_{D600} (msec)</th>
<th>RMP (mV)</th>
<th>AMP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBP</td>
<td>SHAM</td>
<td>HBP</td>
</tr>
<tr>
<td>Control</td>
<td>40.7 ± 10.4</td>
<td>19.0 ± 7.7</td>
<td>63.7 ± 20.7</td>
</tr>
<tr>
<td>D600</td>
<td>30.8 ± 9.0</td>
<td>15.6 ± 5.2</td>
<td>51.0 ± 21.8</td>
</tr>
</tbody>
</table>

Data in the upper part of the table are presented as mean ± SD. In the lower part of the table, group refers to the differences between HBP and SHAM preparations independent of treatment; treatment refers to differences produced by the treatment (D600: 0.5 μg/ml) independent of whether the preparation was from an HBP or SHAM animal; interaction refers to a differentially greater treatment effect on either the HBP or SHAM preparation. F = F value and P = probability value for HBP vs. SHAM. Degrees of freedom for F test = 1 and 8.

Effects of TEA

The effects of TEA (15–20 mM), an outward K⁺ channel-blocking agent, were investigated in eight preparations (four HBP, four SHAM). The results of these experiments are summarized in Table 8 and show: (1) that there was a significant group difference in APD_{D600}, a borderline significant group difference in APD_{D75}, and no significant group difference in RMP or AMP; (2) that treatment with TEA induced highly significant increases in APD_{D600} and APD_{D75} as well as significant decreases in RMP and AMP in both HBP and SHAM AP; and (3) that there was no significant differential effect of TEA treatment on AP parameters of HBP and SHAM animals.

Figure 6 shows records from an additional experiment with 30 mM TEA. Panels A and D show that exposure to TEA prolongs repolarization in both HBP and SHAM AP. Panels B and E show that exposure to TEA at a slower sweep speed to permit the entire course of repolarization to be seen. Panels C and F show that during repolarization with normal Tyrode’s solution, the lengthening of repolarization induced by TEA is at least partly reversible. During exposure to drug-free solution, both HBP and SHAM AP return toward their pretreatment configurations.

Discussion

The results presented in this paper show that HBP AP are consistently and substantially longer than SHAM AP, despite the fact that they do not differ significantly with respect to RMP, AMP, or OS. The AP lengthening in HBP fibers is presumed to be associated with myocardial hypertrophy induced by renal hypertension because: (1) HBP rats showed a 37% increase in mean HW as compared to SHAM rats in the face of comparable values for mean BW; (2) electron microscopic studies of myocardium from renal HBP rats show an increase in average cell size which indicates the presence of hypertrophy (Wendt-Gallitelli et al., 1978; Loud et al., 1978).

It is possible that the AP prolongation seen in rats with renal hypertension could be due, at least in part, to a humoral agent associated with renal hypertension. For example, angiotension II, which is elevated in renal hypertension, has been shown to increase APD in rabbit atrium and ventricle (Bonnardeaux and Regoli, 1974). However, the in-
crease in APD in HBP AP persists for hours in a tissue bath perfused with Tyrode’s solution free of angiotension II or other humoral agents. This result suggests that the action of a humoral agent is unlikely to be the primary cause of prolonged AP in hypertrophied myocardium.

A recently reported study (Gulch et al., 1979) using the same renal HBP rat model describes a similar striking prolongation in HBP AP without significant changes in other AP parameters. Variable degrees of generally less marked AP prolongation also have been reported in cats with RVH (Gelband and Bassett, 1973; Tritthart et al., 1975; Ten Eick, 1977, 1978) and in SHR (Hayashi and Shibata, 1974; Heller et al., 1978; Heller and Stauffer, 1979) and DOCA-HBP rats (Heller and Stauffer, 1979).

Gulch et al. (1979) also studied the effects of raising external K+ (13 mM) in Tyrode’s with normal [Ca2+]o (2.2 mM) and reduced [Ca2+]o (0.55 mM) and tetrodotoxin in normal Tyrode’s and in Tyrode’s with reduced [Na+]o, (65 mM) and [Ca2+]o (0.55 mM). These studies appear to have been performed at a

### Table 8  Effects of TEA on HBP and SHAM AP Parameters

<table>
<thead>
<tr>
<th></th>
<th>HBP</th>
<th>SHAM</th>
<th>HBP</th>
<th>SHAM</th>
<th>HBP</th>
<th>SHAM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APD50 (msec)</td>
<td>APD75 (msec)</td>
<td>RMP (mV)</td>
<td>AMP (mV)</td>
<td>RMP (mV)</td>
<td>AMP (mV)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>51.8 ± 15.8</td>
<td>23.8 ± 4.5</td>
<td>89.8 ± 27.8</td>
<td>43.5 ± 6.2</td>
<td>78.5 ± 2.3</td>
<td>85.3 ± 5.0</td>
</tr>
<tr>
<td>TEA</td>
<td>92.0 ± 24.3</td>
<td>65.5 ± 19.7</td>
<td>133.5 ± 32.2</td>
<td>103.0 ± 27.0</td>
<td>73.5 ± 3.1</td>
<td>80.3 ± 8.0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Group</td>
<td>6.09</td>
<td>0.049</td>
<td>5.68</td>
<td>0.055</td>
<td>5.05</td>
<td>0.066</td>
</tr>
<tr>
<td>Treatment</td>
<td>49.82</td>
<td>0.000</td>
<td>43.16</td>
<td>0.001</td>
<td>6.06</td>
<td>0.049</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.02</td>
<td>0.902</td>
<td>1.00</td>
<td>0.355</td>
<td>0.00</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Data in the upper part of the table are presented as mean ± SD. In the lower part of the table, group refers to the differences between HBP and SHAM preparations independent of treatment; treatment refers to differences produced by the treatment (15-20 mM TEA) independent of whether the preparation was from an HBP or SHAM animal; interaction refers to a differentially greater treatment effect on either the HBP or SHAM preparation. F = F value and P = probability value for HBP vs. SHAM. Degrees of freedom for F test = 1 and 6.
lower temperature (25°C) and at a slower stimulation rate (cycle length = 5000 msec) than in the present study. Furthermore, Gulch et al. (1979) did not study the effects of raising [Ca\(^{2+}\)]\(_o\), other divalent cations, or blocking agents as reported in our study. Gulch et al. (1979) interpreted their results as showing that none of their treatments produces appreciable shortening of HBP AP, but this may be partly a consequence of the method they use to compare AP recorded before and after treatment. This method involves arbitrarily superimposing on the same baseline "AP" reconstructed from mean values. In any case, these investigators interpret their results as suggesting that HBP AP are prolonged because of slowed inactivation of the slow inward current.

Our results show that, in general, the responses of HBP and SHAM AP to various treatments differ in a quantitative rather than qualitative fashion. Quantitatively significant differences in responsiveness of HBP AP, as compared to SHAM AP, were found for some treatments but not for others. For example, exposure to Ca\(^{2+}\)-containing or low-Na\(^+\) Tyrode's solution produced a differentially greater decrease in APD\(_{50}\) and APD\(_{75}\) in HBP AP than in SHAM AP. Treatment with D600 also produced differential shortening of HBP AP, but its effect was limited to APD\(_{50}\). In contrast, exposure to Sr\(^{2+}\)-containing and TEA-containing Tyrode's solution produced an increase in APD\(_{50}\) and APD\(_{75}\), but the lengthening effect was not differentially greater in HBP than SHAM SP. Treatment with Ca\(^{2+}\)-free Tyrode's solution had little effect on APD in either HBP or SHAM animals. None of the treatments had a significant differential action on RMP or AMP in HBP as compared to SHAM AP.

The shortening effect of high [Ca\(^{2+}\)]\(_o\) is similar to that reported by Hoffman and Suckling (1956) in canine atrial, ventricular, and Purkinje fibers exposed to elevated [Ca\(^{2+}\)]. The finding that the shortening effect of high [Ca\(^{2+}\)] is not mimicked by high [Mg\(^{2+}\)] or [Sr\(^{2+}\)], indicates that this action is unique to Ca\(^{2+}\) as opposed to a nonspecific effect of charge neutralization by divalent cations.

The differential shortening effect of low-Na\(^+\) Tyrode's solution on HBP AP is similar to that described by Langer et al. (1975) for AP recorded from 1-day-old as compared to adult rats. Langer et al. (1975) suggested that neonatal rat AP were longer and more susceptible to shortening during exposure to low [Na\(^+\)], because of the presence of slow Na\(^+\) channels that disappear as the animals mature. On the other hand, Gulch et al. (1979) reported that treatment with low (50%) [Na\(^+\)], Tyrode's solution produced lengthening of both HBP AP and control AP. However, in their study, Gulch et al. (1979) reduced not only [Na\(^+\)], but also [Ca\(^{2+}\)] (0.55 mm). Therefore, their experimental conditions were different from ours and those of Langer et al. (1975) in which [Ca\(^{2+}\)] was not reduced simultaneously. This may account for the difference in reported results, although Langer et al. (1975) state that the shortening action of low [Na\(^+\)], was not significantly altered by the simultaneous reduction of [Ca\(^{2+}\)], to 25% of control.

Our findings that both raised [Ca\(^{2+}\)], and lowered [Na\(^+\)], produce differentially greater shortening in HBP AP than in SHAM AP suggest a number of possible mechanisms that might account for prolongation of HBP AP. On one hand, high [Ca\(^{2+}\)], could produce shortening of HBP AP by restoring toward normal a Ca\(^{2+}\)-dependent outward current (Siegelbaum et al., 1977) that is reduced in hypertrophied myocardium. Against this possibility are our results with TEA, which is considered to block outward K\(^+\) currents in a variety of excitable cells (Armstrong, 1975), including cardiac Purkinje fibers (Kenyon and Gibbons, 1979). If one assumes that TEA has a similar action on rat myocardium, the failure of TEA to produce differential lengthening in HBP AP suggests that a decrease in TEA-sensitive outward current is not primarily responsible for prolongation of HBP AP. It is possible, of course, that a Ca\(^{2+}\)-induced outward current that is insensitive to TEA inhibition is reduced in hypertrophied myocardium.

On the other hand, the differential shortening action of high [Ca\(^{2+}\)], on HBP AP could be the result of accelerated inactivation of a Ca\(^{2+}\)-inactivated inward current (Kohlhardt et al., 1975; Brehm and Eckert, 1978) in hypertrophied myocardium. Our results with Sr\(^{2+}\)-containing Tyrode's solution are in favor of this view. Exposure to Sr\(^{2+}\)-containing, Ca\(^{2+}\)-free Tyrode's solution caused lengthening of both HBP and SHAM AP, but this treatment failed to show differential action. These results suggest that Sr\(^{2+}\) is able to pass through the slow channels in rat myocardium (Besseau and Gargouil, 1969; Mainwood and McGuigan, 1975). The lengthening action is presumably the result of Sr\(^{2+}\) entry, which is not as effective as Ca\(^{2+}\) entry in producing inactivation of the slow channel (Brehm and Eckert, 1978). Consequently, Sr\(^{2+}\) fails to produce a differential shortening of HBP AP. Thus, the results with Sr\(^{2+}\) are consistent with the view that prolongation of HBP AP may be due to slowed inactivation of a Ca\(^{2+}\)-inactivated inward current.

The differential shortening action of low [Na\(^+\)], on HBP AP could be an indirect result of partial inhibition of the Na\(^+\)-Ca\(^{2+}\) exchange system (Jundt et al., 1975) leading to increased internal Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)). The increased [Ca\(^{2+}\)]\(_i\) so produced could, in turn, accelerate repolarization by the mechanism suggested above with regard to high [Ca\(^{2+}\)]. Alternatively, it is possible that a new slow channel capable of carrying Na\(^+\) and regulated (e.g., inhibited or blocked) by Ca\(^{2+}\) may develop in hypertrophied myocardium. Channels of this kind should produce the differential shortening of HBP AP we have found in either high [Ca\(^{2+}\)], or low...
Another possibility is that existing channels of normal fibers may be altered in hypertrophied myocardium so as to develop inward rectifying properties as suggested by Ten Eick et al. (1978). According to this view, differential shortening of HBP AP induced by high [Ca\textsuperscript{2+}], and low [Na\textsuperscript{+}], would be caused by these treatments somehow reducing the inward rectifying properties of the channels involved.

Finally, prolongation of HBP AP could be the result of altered electrotonic interactions between fibers in hypertrophied myocardium. In this case, the differential shortening action of high [Ca\textsuperscript{2+}] and low [Na\textsuperscript{+}] on HBP AP presumably would be the result of these treatments altering the electrotonic interactions responsible for prolongation of HBP AP in hypertrophied myocardium.

Prolongation of HBP AP may represent a compensatory mechanism for preserving the contractile strength of hypertrophied myocardium. The longer AP presumably enhances contraction by providing a longer signal for activation of contraction. The contractile alterations associated with myocardial hypertrophy in the renal HBP rat (increased peak force, increased time-to-peak force and slowed relaxation) are consistent with this view (Jacob et al., 1977; Strobeck et al., 1980; Gulch et al., 1979).

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Computer Simulation of Arrhythmias in a Network of Coupled Excitable Elements

FRANS J.L. VAN CAPELLE AND DIRK DURRER

SUMMARY Arrhythmias were simulated in sheets or cables, consisting of coupled excitable elements, which were characterized by a simple regenerative mechanism. The geometry of the network, the amount of coupling among individual elements, and the properties of the elements relating to excitability, automaticity, and duration of the refractory period could be adjusted arbitrarily in an interactive computer program. When a critical amount of coupling was present between automatic and non-automatic cells, sustained repetitive activity could be initiated and stopped by stimulation of the elements. Using this mechanism, it was also possible to evoke reciprocal activity in a one-dimensional cable. In uniform sheets of coupled elements, circus movement of the activation front could be evoked. The presence of an obstacle or dispersion of the refractory periods of the elements was not a prerequisite for the initiation of circus movements. The vortex of circus movements in the homogeneous sheets consisted of elements which were inactivated by depolarizing currents from the circulating wavefront. In sheets of sufficient size, multiple vortices could be present. CIRC Res 47: 454-466, 1980

WE used computer simulation to study the possible role of spatial interaction among excitable elements in the mechanisms underlying cardiac arrhythmias, of both the focal and the reentrant type. Intuitively, the mechanism of focal tachycardias is thought to reside mainly in the ionic properties of the cell membrane, whereas the involvement of many interacting cells is a prerequisite for reentrant arrhythmias. We were interested in whether it would be possible to evoke both focal and reentrant arrhythmias in a synthetic sheet consisting of a primitive kind of excitable element, and whether the influence the elements exert on each other would play an important role in the genesis and maintenance of these arrhythmias. As it turns out, many of the features of clinical and experimental tachycardias could be simulated rather easily in such a model, and the results suggest that the role of spatial interaction among cardiac cells may be of considerable importance in both types of arrhythmia.

Although many model studies of impulse propagation have been published, relatively few of them deal with sheets consisting of large numbers of elements. Notable exceptions are an early computer study (Moe et al., 1964), demonstrating the importance of dispersion of the duration of the refractory periods of the elements in the mechanism of fibrillation, and an elegant hybrid study (Gul’ko and Petrov, 1972) on circus movement of the activation front in homogeneous sheets. The reason for this scantiness is that long trains of action potentials have to be calculated for many elements simultaneously in this type of study, and this requires an enormous amount of computation, even on a large computer. We dealt with this problem using a simplified model for the individual excitable elements, and interactive computer techniques. With the help of interactive computer programs, irrelevant solutions may be skipped and interesting episodes may be repeated immediately, using other parameter settings, or displaying the signals of different elements. The simulation is performed very much as in an actual experiment. For instance, before setting the stimulus strength, one has to determine stimulus threshold levels, to set the stimulus sequence, and to make decisions about the location of the stimuli. The computer dialogue is formulated in the usual electrophysiological terminology. In accordance with this practice, electrophysiological terms such as tachycardia, refractory period, etc., will be applied freely in the present text. Although it may seem inappropriate to refer to a computer as having a tachycardia, we believe that the practical advan...
Characteristics of action potentials of hypertrophied myocardium from rats with renal hypertension.

R S Aronson

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