Effects of Rapid Stimulation on the Transmembrane Action Potentials of Rabbit Sinus Node Pacemaker Cells

ITSUO KODAMA, JUNKI GOTO, SHIGEYUKI ANDO, JUNJI TOYAMA, AND KAZUO YAMADA

SUMMARY We studied the mechanism of post-overdrive suppression in superfused rabbit sinus node pacemaker cells. Small specimens of sinus node tissue isolated from rabbit hearts were driven at a fast rate (overdrive) for 10-120 seconds using single sucrose gap methods. During the control perfusion (35°C Tyrode's solution), overdrive caused a progressive decrease in maximum diastolic potential (MDP), overshoot (OS), and maximum rate of depolarization at phase 0 \([dV/dt]_{\text{max}}\). After cessation of the overdrive, the rate of diastolic depolarization decreased, and the spontaneous activity was suppressed temporarily (post-overdrive suppression). MDP, OS, \([dV/dt]_{\text{max}}\), and the spontaneous activity returned within a few seconds to the level observed before overdrive. Atropine \((2 \times 10^{-7} \text{ g/ml})\) did not influence the effects of overdrive. After ouabain administration \((3 \times 10^{-7} \text{ g/ml})\) or in low temperature perfusate \((25^\circ C)\), the effects of overdrive were accentuated, and a marked suppression of spontaneous activity with a long pause of over several seconds was seen following the overdrive. These results suggest that the post-overdrive suppression of sinus node is attributable, at least in part, to ionic shifts following overdrive, and may be potentiated by metabolic dysfunction of pacemaker cells. Circ Res 46: 90-99, 1980

"Overdrive suppression tests" through the use of rapid atrial pacing have been employed extensively in the evaluation of patients with sinus node dysfunction (Mandel et al., 1972; Narula et al., 1972; Ferrer, 1973; Gupta et al., 1974; Strauss et al., 1976; Breithardt et al., 1977; Jordan et al., 1978). However, in spite of many in situ and in vitro studies, much remains to be clarified as to the underlying mechanism of sinus node post-overdrive suppression (Vassalle, 1977). West and collaborators (West, 1961; Amory and West, 1962; Vincenzi and West, 1963) reported that repetitive electrical stimulation of isolated rabbit sinus node tissue was followed by a period of suppression of spontaneous activity accompanied by membrane hyperpolarization. Since both the suppression and the hyperpolarization were potentiated by physostigmine and abolished by atropine, they attributed the negative chronotropic effect of the repetitive electrical stimuli to the release of endogenous acetylcholine from the sinus node tissue. Lu et al. (1965) obtained similar results in their experiments using isolated cat sinus node preparations. However, the finding that atropine did not abolish completely the post-overdrive suppression led them to the conclusion that, although acetylcholine release from the cardiac tissue plays an important role in sinus node post-overdrive suppression, other factors are involved in this phenomenon. These factors could include conduction failure within the sinus node or ionic shifts, e.g., those resulting from augmented \(K^+\) efflux and/or \(Na^+\) or \(Ca^{2+}\) influx. This hypothesis was supported by the in situ studies of Lange (1965).
Recently, some experimental results of Noma and Irisawa (1974, 1975) suggest that the activity of the electrogenic sodium pump of the cell membrane affects the transmembrane action potentials of sinus node pacemaker cells, and it is assumed that the post-overdrive suppression of the sinus node may be due partly to the activation of the electrogenic sodium pump (Irisawa, 1978), which was proposed as a major mechanism responsible for the post-overdrive suppression in Purkinje fibers (Vassalle, 1970).

In the previous studies on the isolated preparation including the whole sinus node tissue, it was found to be very difficult to elucidate the effects of overdrive on the action potentials of sinus node pacemaker cells for the following reasons. The conduction of excitation can be disturbed easily in the transitional portion between atrial muscle and sinus node as well as within the node (Lu et al., 1965; Strauss et al., 1968; Han et al., 1968; Bonke et al., 1971; Strauss and Bigger, 1972; Brooks and Lu, 1972). Thus, impulses from the stimulating electrodes sometimes fail to fire the pacemaker cells (entrance block) or impulses emerging from the pacemaking site may be blocked without eliciting atrial excitation (exit block). The pacemaking site often shifts even spontaneously within the sinus node, but especially when a premature stimulus or overdrive is applied (Lu et al., 1965; Bonke et al., 1971). This conduction disturbance and pacemaker shift have been reported to be very susceptible to extracellular ionic changes, vagal or sympathetic stimulation, and some drugs, such as ouabain (Brooks and Lu, 1972; Strauss et al., 1968; Bouman et al., 1968; Toda and Shimamoto, 1968; Lu, 1970; Goldberg, 1975; Bouman et al., 1978; Steinbeck et al., 1978), and are very likely to affect both the intensity of the post-overdrive suppression and the transmembrane potential of sinus node cells.

In the present study, we applied stimuli to very

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** Cross-section of the strip dissected from the middle part of the sinus node in a direction perpendicular to the crista terminalis. CT = crista terminalis; SARB = right branch of sinoatrial ring bundle; SN = sinus node; SVC = superior vena cava. Atrial muscle forming the crista terminalis and a part of sinus node tissue near the crista terminalis were removed along the solid line illustrated, and the remaining tissue was employed for experiments. Dotted lines show the approximate position of the two separating rubber membranes when the preparation was mounted in a tissue bath.
small specimens of sinus node tissue, using single sucrose gap methods to avoid conduction disturbance and pacemaker shift within the preparations. We also investigated the effects of overdrive on the membrane action potentials of sinus node pacemaker cells under various experimental conditions. The results obtained suggest that metabolic dysfunction of pacemaker cells plays an important role in enhancing the sinus node post-overdrive suppression.

Methods

Rabbits weighing 1.5–2.0 kg were anesthetized by intravenous injection of thiamylal sodium, 40 mg/kg. The hearts were excised and dissected in aerated Tyrode’s solution. The right atrium was isolated from the remaining part of the heart and was opened by a longitudinal incision in the free wall to expose the endocardial surface. After identifying the sinus node region by recording the transmembrane potential, we dissected a part of the tissue (0.3–0.4 mm in width, 3–4 mm in length), including the middle part of the sinus node, in a direction perpendicular to the crista terminalis. Then, the atrial muscle within the crista terminalis and a part of the sinus node tissue near the crista terminalis (0.5–1.0 mm from the right branch of sinoatrial ring bundle) were removed from the tissue strip, as shown in Figure 1, and discarded. The final sinus node preparation was 0.3 mm wide, 0.2–0.3 mm thick, and 2 mm long. The preparation was transferred to a tissue bath constructed of three compartments (Fig. 2). Each compartment was separated by rubber sheets (70 μm thick) with a hole 0.3 mm in diameter which permitted the preparation to be passed through it. The width of the middle compartment (sucrose gap) was 1.0 mm. The end of the preparation near the crista terminalis occupied the right side compartment (test compartment) and the other end near the superior vena cava, the left side compartment (KCl compartment), as shown in Figure 2. The length of the preparation in the test compartment was about 0.3 mm.

The test compartment was perfused with Tyrode’s solution equilibrated with 100% oxygen. The composition of Tyrode’s solution was as follows (mm): NaCl, 137; KCl, 4.0; CaCl₂, 1.8; MgCl₂, 0.5; dextrose, 5.5; Trizma base, 5.0; HCl, 3.7–3.9. (The solution was titrated with HCl to pH 7.4.) The middle compartment (sucrose gap) was perfused with isotonic sucrose solution. The temperature of the perfusate in the test compartment and sucrose gap usually was maintained at 35°C, but in the low temperature experiments, it was held to 25°C. The KCl compartment was filled with isotonic KCl solution.

The preparations were stimulated by an electrical stimulator (Nihon Kohden SEN 3101) through an Ag-AgCl electrode in the KCl compartment (Fig. 2). A resistor (2 MΩ) in series with the stimulator maintained the stimulus current nearly constant. The driving stimuli rectangular depolarizing pulses of 4–10 msec in width and of about twice diastolic threshold in intensity (1–5 × 10⁻³ A), were applied at a rate ranging from 150/min to 600/min. The duration of drive was varied between 10 and 120 seconds. Glass microelectrodes filled with 3 M KCl and having resistance of 10–15 MΩ were used to record transmembrane potential. The transmembrane potential and its first time derivative obtained by electronic differentiation were displayed on a storage oscilloscope (Tektronix 5103) and on an electromagnetic oscillograph (Yokokawa Electric Works). We employed a virtual ground clamp system (New and Trautwein, 1972) to abolish the extracellular potential change in the test compartment caused by driving stimuli (Fig. 2). This system senses the potential immediately adjacent to the preparation through a glass microelectrode and drives the surrounding bath through an Ag-AgCl electrode as required to hold this extracellular potential always at virtual ground potential.

The following parameters were measured in each experiment: maximum diastolic potential (MDP); maximum rate of depolarization of phase 0 [(dV/dt)ₘₐₓ]; overshoot (OS); cycle length (CL) of spontaneous firing. From the first time derivative of the transmembrane action potentials, (dV/dt)ₘₐₓ was obtained, and CL was determined by measuring the interval between spikes of the first time derivatives.

The preparations were allowed to equilibrate for about 1 hour in the tissue bath before any tests were performed. Seventeen out of 47 preparations that showed unstable or no spontaneous activity during this equilibration period were discarded, and the remaining 30 preparations with regular and stable spontaneous firing were employed for the present experiments. In repetitive tests of overdrive, adequate intervals for recovery were given before additional drives were imposed.

When the effects of overdrive were examined
under the influence of atropine \( (2 \times 10^{-6} \text{ g/ml}) \) or ouabain \( (3 \times 10^{-7} \text{ g/ml}) \), these drugs were added to the reservoir of oxygenated Tyrode's solution to achieve the required concentration.

Statistical analysis was performed using Student's \( t \)-test or correlation coefficient calculation, and significance was established at \( P < 0.05 \). More details of each procedure will be given under Results.

**Results**

**Overdrive under the Control Perfusion**

Effects of overdrive at various rates and of various durations were studied in 30 preparations during control perfusion (35°C oxygenated Tyrode's solution). The parameters of the transmembrane action potential of these spontaneously firing preparations at steady state were as follows (mean ± SD): MDP, \(-65.1 ± 3.5 \text{ mV}\); OS, \(8.5 ± 4.0 \text{ mV}\); \((dV/dt)_{\text{max}}\), \(8.4 ± 3.4 \text{ V/sec}\); CL of spontaneous firing, \(337 ± 46 \text{ msec}\). (The average value for all preparations was based on individual averages of three trials in each preparation.)

The intensity of post-overdrive suppression was related to the rate and the duration of the overdrive. Figure 3 illustrates representative results recorded from the same preparation. The duration of the overdrive in each record was 30 seconds. When the preparation was driven at a rate of 375/min (CL 160 msec, panel A), a progressive decrease in MDP, OS, and \((dV/dt)_{\text{max}}\) was observed during the drive. After the cessation of the drive, spontaneous activity of the preparation was suppressed temporarily, leading to prolongation of the first post-overdrive CL by 40 msec. The CL of spontaneous firing then returned to the pre-overdrive level within 3 seconds. Action potentials immediately after the cessation of the overdrive showed a slight decrease in MDP, OS, and \((dV/dt)_{\text{max}}\) as well as slowing in the rate of diastolic depolarization as compared with steady state action potentials before the drive. As the spontaneous activity resumed, the action potential configuration gradually returned to the control shape in the pre-overdrive period. When the preparation was driven at a rate of 462/min (CL 130 msec, panel B), the changes in transmembrane action potentials during and after the overdrive were accentuated, and more pronounced post-overdrive suppression was obtained. In addition, a slight alternation of action potential amplitude during the overdrive as shown in Figure 3C occurred in most cases, and somewhat less or no post-overdrive suppression was obtained. Although not shown in Figure 3, when the preparations were driven at rates below 230/min, which were only slightly above the spontaneous firing rate, acceleration of spontaneous activity rather than suppression frequently was encountered following the drive.

Post-overdrive suppression usually was accentuated by lengthening the duration of overdrive in the range between 10 and 120 seconds. However, at the higher rates, which produce the alternation of action potential amplitude during the overdrive as shown in Figure 3C occurred in most cases, and somewhat less or no post-overdrive suppression was obtained. Although not shown in Figure 3, when the preparations were driven at rates below 230/min, which were only slightly above the spontaneous firing rate, acceleration of spontaneous activity rather than suppression frequently was encountered following the drive.

**Figure 3** Effects of overdrive for 30 seconds at different rates. Upper trace in each panel is transmembrane action potential, and lower trace is its first time derivative. The numbers below each panel indicate the CL of spontaneous firing before overdrive (on the left) and the first post-overdrive CL (on the right). The preparation was driven at a rate of 375/min (CL 160 msec) in panel A, 462/min (CL 130 msec) in panel B, and 600/min (CL 100 msec) in panel C.
are in agreement with the results of previous investigators (West, 1961; Lu et al., 1965), the change in MDP is quite different from theirs. Thus, we examined the quantitative relationship between the change in MDP and the intensity of post-overdrive suppression. The results are illustrated in Figure 4. Data were obtained from 18 preparations on which 85 overdrive tests of various rates and durations (180–600/min and 10–120 seconds) were performed. In all of these cases, a single microelectrode impalement was maintained throughout the overdrive tests. There was a statistically significant correlation between the decrease in MDP (depolarization) after the last driven beat (in relation to the pre-overdrive value) and the percent increase of the first post-overdrive CL ($r = 0.41, P < 0.01$). A similar significant correlation was obtained between the decrease in MDP following the first post-overdrive spontaneous beat and the intensity of post-overdrive suppression.

Quantitative evaluation of the change in threshold potential after overdrive was not possible in the present study, since recorded action potentials always showed a smooth transition from phase 4 to phase 0.

**Effects of Atropine**

Direct electrical stimulation has been considered to induce the release of endogenous neuromediators (acetylcholine and catecholamine) that serve to elicit the chronotropic phenomenon (West, 1961; Amory and West, 1962; Vincenzi and West, 1963). Accordingly, we examined the effects of atropine in four preparations to elucidate the role of endogenous acetylcholine in the post-overdrive suppression. After performing overdrive tests under the control perfusion, these preparations were superfused with Tyrode’s solution containing atropine ($2 \times 10^{-6}$ g/ml) for 15 minutes, and then the suppressive effect of overdrive was studied again. Both the spontaneous activity of the preparations and the intensity of post-overdrive suppression were little affected by the atropine administration. Thus, the CL of spontaneous firing at steady state was $329 \pm 61$ msec under the control perfusion and $332 \pm 52$ msec after the atropine administration.

### Table 1 Parameters of Transmembrane Action Potential of Six Preparations during Control Perfusion and after Ouabain ($3 \times 10^{-7}$ g/ml) Administration

<table>
<thead>
<tr>
<th>Preparation no.</th>
<th>MDP (mV)</th>
<th>OS (mV)</th>
<th>(dV/dt)$_{max}$ (V/sec)</th>
<th>CL (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68.0</td>
<td>64.0</td>
<td>8.2</td>
<td>368</td>
</tr>
<tr>
<td>2</td>
<td>65.8</td>
<td>58.8</td>
<td>10.6</td>
<td>380</td>
</tr>
<tr>
<td>3</td>
<td>70.0</td>
<td>59.3</td>
<td>8.3</td>
<td>363</td>
</tr>
<tr>
<td>4</td>
<td>64.0</td>
<td>53.6</td>
<td>7.2</td>
<td>443</td>
</tr>
<tr>
<td>5</td>
<td>67.1</td>
<td>55.5</td>
<td>9.8</td>
<td>340</td>
</tr>
<tr>
<td>6</td>
<td>68.4</td>
<td>58.0</td>
<td>9.2</td>
<td>363</td>
</tr>
<tr>
<td>Average $\pm$ SD</td>
<td>67.2 $\pm$ 2.1</td>
<td>58.2 $\pm$ 3.6*</td>
<td>8.9 $\pm$ 1.2</td>
<td>356 $\pm$ 59</td>
</tr>
</tbody>
</table>

* Significantly different from the value under the control perfusion ($P < 0.05$).
Effects of Overdrive on Sinus Node Pacemaker Cells/Kodama et al.

After atropine administration (mean ± SD in four preparations). With overdrive for 30 seconds at a rate of about twice the spontaneous firing rate, the first post-overdrive CL was prolonged by 42 ± 36 msec under the control perfusion and 49 ± 47 msec after atropine administration (mean ± SD in four preparations). Likewise, the changes in transmembrane action potential during and after overdrive were not influenced by atropine administration.

Effects of Ouabain

In our preliminary experiments, a continuous perfusion with ouabain caused a progressive change in the transmembrane action potential of the sinus node cells, and steady state could not be obtained during the perfusion. Therefore, we examined the effects of ouabain on the post-overdrive suppression in the following way. After performing overdrive tests in the normal Tyrode’s solution (control perfusion), the preparations were superfused with a solution containing ouabain (3 × 10⁻⁷ g/ml) for 30 minutes. Then the perfusate was returned to the normal Tyrode’s solution. During the reperfusion with the normal Tyrode’s solution, the resulting state from the initial ouabain administration remained almost constant for about 30 minutes (and was followed by gradual recovery to the control values). Effects of ouabain on the post-overdrive suppression were studied at 5-10 minutes after returning to the normal Tyrode’s solution.

Ouabain-induced changes in the transmembrane action potential measured immediately before the overdrive tests are summarized in Table 1. The rate of overdrive was about twice the spontaneous firing rate for each experimental condition, and the duration of overdrive was 30 seconds. A typical example is shown in Figure 5. Records in panel A and B were obtained from the same preparation. During the control perfusion (panel A), the first post-overdrive CL prolonged by only 6 msec. After the administration of ouabain (panel B), overdrive was followed by a marked suppression of spontaneous activity with a long pause of more than 3 seconds. In this preparation, it took about 8 seconds for the suppressed spontaneous activity to return to the pre-overdrive level. A similar potentiation of the post-overdrive suppression was noted in the other five preparations (Table 2).

The changes in the transmembrane action potential were analyzed by comparing the pre-overdrive values to the post-overdrive values. The results are summarized in Table 2. The changes in the transmembrane action potential were evaluated by measuring the changes in peak action potential (AMDP), action potential duration at different voltages (AOS), and action potential rate (A(dV/dt)max) during and after overdrive.

Table 2: Change in Parameters of Transmembrane Action Potential following Overdrive

<table>
<thead>
<tr>
<th>Preparation no.</th>
<th>Control</th>
<th>Ouabain</th>
<th>Control</th>
<th>Ouabain</th>
<th>Control</th>
<th>Ouabain</th>
<th>Control</th>
<th>Ouabain</th>
<th>Control</th>
<th>Ouabain</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔMDP (mV)</td>
<td>-2.0</td>
<td>-4.0</td>
<td>-1.7</td>
<td>-2.6</td>
<td>-1.5</td>
<td>-2.4</td>
<td>+6</td>
<td>+3130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔOS (mV)</td>
<td>-0.2</td>
<td>-2.0</td>
<td>-1.2</td>
<td>-8.0</td>
<td>+1.2</td>
<td>+1.5</td>
<td>+14</td>
<td>+1062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ(dV/dt)max (V/sec)</td>
<td>+2.6</td>
<td>+3.2</td>
<td>-0.6</td>
<td>-1.8</td>
<td>-2.1</td>
<td>-2.0</td>
<td>+92</td>
<td>+3870</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔCL (msec)</td>
<td>-1.1</td>
<td>-3.2</td>
<td>-3.2</td>
<td>-5.3</td>
<td>-1.9</td>
<td>-5.3</td>
<td>+7</td>
<td>+2350</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average ± SD: -0.6 ± 0.9, -3.5 ± 1.1*, -2.2 ± 1.0, -3.8 ± 2.2*, -1.1 ± 1.2, -2.5 ± 1.4*, +30 ± 35, +2601 ± 1426*

Overdrive tests were performed during the control perfusion as well as after ouabain administration, and the change in each parameter following the overdrive, in relation to the pre-overdrive value, was obtained. ΔMDP = change in MDP following the last driven beat; ΔOS = change in OS of the first post-overdrive spontaneous beat; Δ(dV/dt)max = change in (dV/dt)max of the first post-overdrive spontaneous beat; ΔCL = change in the first post-overdrive CL.

*Significantly different from the value under the control perfusion (P < 0.05).

Figure 5: Effects of ouabain on the post-overdrive suppression. The rate of overdrive was 333/min (CL 180 msec) in panel A (control perfusion) and 300/min (CL 200 msec) in panel B (after ouabain administration). Numbers below each panel represent the CL before overdrive and the first post-overdrive CL.
Table 3  Parameters of Transmembrane Action Potential of Five Preparations during Control Perfusion (35°C) and in Low Temperature Perfusate (25°C)

<table>
<thead>
<tr>
<th>Preparation no.</th>
<th>MDP (mV)</th>
<th>OS (mV)</th>
<th>(dV/dt)_{max} (V/sec)</th>
<th>CL (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Low temp</td>
<td>Control</td>
<td>Low temp</td>
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<tr>
<td>1</td>
<td>62.8</td>
<td>55.2</td>
<td>8.2</td>
<td>5.2</td>
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<tr>
<td>2</td>
<td>65.3</td>
<td>62.0</td>
<td>7.1</td>
<td>1.5</td>
</tr>
<tr>
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<td>64.0</td>
<td>59.0</td>
<td>9.7</td>
<td>8.6</td>
</tr>
<tr>
<td>4</td>
<td>67.7</td>
<td>64.7</td>
<td>7.0</td>
<td>4.9</td>
</tr>
<tr>
<td>5</td>
<td>73.4</td>
<td>66.1</td>
<td>12.0</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Average ± SD 66.6 ± 4.2 61.4 ± 3.9* 8.8 ± 2.1 5.3 ± 2.6* 8.7 ± 1.7 4.2 ± 1.0* 328 ± 42 633 ± 55*

Abbreviations and symbols are the same as in Table 1.

* Significantly different from the value under the control perfusion (P < 0.05).

tial following overdrive [the decrease in MDP, OS, and (dV/dt)_{max}] also were accentuated after ouabain administration. Quantitative data obtained from each preparation are presented in Table 2. After ouabain administration, the diastolic depolarization immediately after the cessation of overdrive was composed of an initial portion having a relatively steeper slope and a later portion that was hardly sloped at all. In addition, fine subthreshold oscillatory potentials were recorded frequently (Fig. 5).

These effects of ouabain on the post-overdrive suppression were not affected by atropine (2 x 10^{-6} g/ml).

Low Temperature Perfusate

Effects of low temperature were examined in five preparations. The changes in the parameters of transmembrane action potential caused by lowering the temperature from 35°C to 25°C are summarized in Table 3. In the low-temperature perfusate, although the CL of spontaneous firing lengthened to about twice the control values, a stable and regular spontaneous activity still was maintained in each preparation.

Overdrive tests were performed in both the control and low temperature perfusate, and results are shown comparatively in Table 4. The rate of overdrive was about twice the spontaneous firing rate in the respective experimental conditions, and its duration was 30 seconds. As shown in Figure 6 and Table 4, overdrive in the low temperature perfusate caused a marked post-overdrive suppression which was never seen under the control perfusion.

Changes in transmembrane action potentials during and after overdrive in the low temperature perfusate were qualitatively similar to those after ouabain administration. However, OS of the first post-overdrive spontaneous beat following very long pauses of over several seconds was sometimes larger than that before the overdrive (Fig. 6, Table 4).

In two preparations, the longest CL (pause) was

Table 4  Change in Parameters of Transmembrane Action Potential following Overdrive

<table>
<thead>
<tr>
<th>Preparation no.</th>
<th>ΔMDP (mV)</th>
<th>ΔOS (mV)</th>
<th>Δ(dV/dt)_{max} (V/sec)</th>
<th>ΔCL (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Low temp</td>
<td>Control</td>
<td>Low temp</td>
</tr>
<tr>
<td>1</td>
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<td>-0.7</td>
<td>+3.4</td>
</tr>
<tr>
<td>2</td>
<td>-1.0</td>
<td>-3.4</td>
<td>-1.2</td>
<td>-2.8</td>
</tr>
<tr>
<td>3</td>
<td>-1.9</td>
<td>-3.3</td>
<td>-4.2</td>
<td>+4.0</td>
</tr>
<tr>
<td>4</td>
<td>-1.3</td>
<td>-2.7</td>
<td>-2.1</td>
<td>-4.4</td>
</tr>
<tr>
<td>5</td>
<td>+0.2</td>
<td>-2.0</td>
<td>-0.7</td>
<td>-3.2</td>
</tr>
</tbody>
</table>

Average ± SD -0.9 ± 0.5 -2.8 ± 0.6* -1.8 ± 1.5 -0.6 ± 4.0 -1.6 ± 0.8 -1.9 ± 0.7 +50 ± 42 +8,030 ± 6,213*

Abbreviations and symbols are the same as in Table 2.

Since this case showed the "secondary pause," the value was obtained from the second post-overdrive CL (the same case as shown in Fig. 7).

* Significantly different from the value under the control perfusion (P < 0.05).
EFFECTS OF OVERDRIVE ON SINUS NODE PACEMAKER CELLS/Kodama et al. 97

We studied the effects of rapid stimulation (overdrive) on very small specimens of sinus node tissue to avoid pacemaker shift and conduction disturbance within the preparations. Thus, the size of preparations in the test compartment was approximately half, or less, of the length constant of sinus nodes evaluated by previous investigators (Bonke, 1973; Seyama, 1976). Noma and Irisawa (1976) reported that the spatial homogeneity of transmembrane potential was maintained within sinus node preparations having almost the same size as ours. Although we did not confirm the potential distribution within the preparation in the test compartment, the following findings may indicate that nearly uniform distribution of transmembrane potential was maintained and no conduction occurred within the preparations. (1) When the preparations were allowed to beat spontaneously, the recorded transmembrane action potentials always showed a smooth transition from phase 4 to phase 0, as is characteristic of true pacemaker cells. (2) No changes in transmembrane action potentials suggesting a shift of pacemaker site within the preparations were observed.

The values of MDP, OS, and (dV/dt)_max recorded at steady state during the control perfusion in the present study are a little larger than the previously reported values of the true sinus node pacemaker cells (Brooks and Lu, 1972; Strauss et al., 1977; Irisawa, 1978). Thus, it might be argued that the preparations used in the present study included the border area of the sinus node near the crista ter-

Discussion

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The values of MDP, OS, and (dV/dt)_max recorded at steady state during the control perfusion in the present study are a little larger than the previously reported values of the true sinus node pacemaker cells (Brooks and Lu, 1972; Strauss et al., 1977; Irisawa, 1978). Thus, it might be argued that the preparations used in the present study included the border area of the sinus node near the crista ter-

obtained consistently following the last driven beat. However, in the other three preparations, it was obtained after either the last driven beat or the first post-overdrive spontaneous beat (secondary pause). The first post-overdrive CL in the cases that showed secondary pause was prolonged by only 10-180 msec (Fig. 7).

FIGURE 7 Secondary pause in the low temperature perfusate. The rate of overdrive was 240/min (CL 250 msec), and its duration was 30 seconds. Numbers below each record indicate the CL before overdrive and after its cessation.

Since the experimental reports by West et al. (West, 1961; Amory and West, 1962; Vincenzi and West, 1963), the major mechanism responsible for the post-overdrive suppression of sinus node has been attributed to the release of endogenous acetylcholine from the cardiac tissue elicited by the electrical stimulation. Nevertheless, the post-overdrive suppression of the sinus node pacemaker cells observed in the present study cannot be explained by this hypothesis for the following reasons. Overdrive in the present experiments caused a decrease in MDP (depolarization), whereas the previous investigators reported that rapid electrical stimulation applied to the preparations led to hyperpolarization of sinus node cells (West, 1961; Amory and West, 1962). Under control perfusion, there was a significant correlation between the magnitude of the depolarization and the intensity of the post-overdrive suppression. In addition, atropine exerted little effect on the post-overdrive suppression. Furthermore, overdrive at a very high rate or of long duration, when accompanied by marked alternation of action potential amplitude, was followed by the least post-overdrive suppression. This finding would indicate that the intensity of post-overdrive suppression is related closely to the number of action potentials elicited, not to the number of electrical stimuli applied.

The contribution of an electrogenic sodium pump, which is supposed to be a major mechanism responsible for the post-overdrive suppression in Purkinje fibers (Vassalle, 1970), is also unlikely in the sinus node pacemaker cells, because in our experiments, sinus node pacemaker cells showed depolarization instead of hyperpolarization after overdrive; also, ouabain, an inhibitor of the sodium pump of the cell membrane (Schwartz, 1974), potentiated the post-overdrive suppression. The difference between the effects of overdrive in Purkinje fibers and in sinus node cells might be explained as follows, in terms of activation of the electrogenic sodium pump. Sodium inward current at the depolarizing phase of transmembrane action potentials in sinus node cells is much less than in Purkinje fibers, and also sinus node cells have a relatively high background sodium current (Noma and Irisawa, 1976; Strauss et al., 1977; Irisawa, 1978). Thus, the increment of sodium influx during overdrive could be insufficient for the activation of the elec-
togenic sodium pump which causes hyperpolarization and modifies the pacemaker activity. An alternative explanation might be that the short circuiting effect (Vassalle, 1970; Noma and Irisawa, 1975) of Cl⁻ or K⁺ on the electrogenic Na⁺ extrusion by the sodium pump is more pronounced in sinus node cells than in Purkinje fibers.

The post-overdrive suppression in the present experiments may be attributed to some ionic shifts following the overdrive. Since the number of action potentials per unit time is higher during the overdrive, the efflux of K⁺ and influx of Ca²⁺ and/or Na⁺ per unit time would increase correspondingly (Noma and Irisawa, 1976; Strauss et al., 1977; Irisawa, 1978). As a consequence, extracellular accumulation of K⁺ adjacent to the cell membrane or an increase in intracellular Ca²⁺ and/or Na⁺ concentration could occur. Among these ionic shifts, potassium accumulation outside the cell membrane does not seem to contribute to the post-overdrive suppression in the sinus node cells, since the spontaneous activity of the sinus node is relatively insensitive to the higher extracellular K⁺ concentration (Brooks and Lu, 1972). Lu (1970) reported that increasing the extracellular K⁺ concentration from 2.7 to 8.1 mM caused a slight acceleration of sinus node spontaneous activity rather than suppression. We also examined the effects of higher extracellular K⁺ concentration using three preparations employed in the present study and found that no significant change occurred in the spontaneous CL of these preparations after increasing the K⁺ concentration in the perfusate from 4.0 to 8.0 mM.

On the contrary, an intracellular accumulation of Ca²⁺ and/or Na⁺ is likely to play a more important role in the post-overdrive suppression for the following reasons. The decrease in OS and (dV/dt)_{max}, which was observed in the present study as the characteristic change in transmembrane action potentials following overdrive, may have resulted from decreased inward current of Ca²⁺ or Na⁺ secondary to the reduction of the concentration gradient of these cations across the cell membrane. Furthermore, the post-overdrive suppression apparently was accentuated by either ouabain administration or lowering the temperature of the perfusate. Both of these experimental conditions may have inhibited active ion transport (Woodbury, 1962; Schwartz, 1974) of the sinus node cells and made possible accumulation of Ca²⁺ or Na⁺ during the overdrive sufficient to cause a marked post-overdrive suppression.

The decrease in MDP (depolarization) with overdrive presumably may be related to the above-mentioned ionic shifts. However, it is impossible from the present data to determine the exact nature of the ionic shifts responsible for this phenomenon.

The methods we used for the present study have a definite advantage over previous ones (West, 1961; Lu et al., 1965) in terms of examining the effects of overdrive on the transmembrane potential of pacemaker cells without any disturbance by pacemaker shift or conduction block. However, some physiological properties of the sinus node, especially those related to innervation, may be lost in such small preparations. Thus, the present results might only reveal direct effects of overdrive on the sinus node pacemaker cells. On the other hand, net effects of overdrive in the in situ heart presumably include indirect effects through neuromediators as well as the direct effects (Lange, 1965; Jordan et al., 1977; Jordan et al., 1978). The difference between our results and those reported by West (1961) or Lu et al. (1965) might be due partly to the effects of overdrive through these neuromediators. Our method of driving small specimens of sinus node tissue by using a sucrose gap technique might cause much less release of neuromediators from the preparations than the previous methods, in which stimuli were applied through extracellular electrodes to a part of the preparations containing almost the whole sinus node.

In patients with "sick sinus syndrome," long pauses (sinus arrest) of over several seconds often are observed after rapid atrial pacing or tachycardia attack. To interpret these long pauses, some hypotheses, such as abnormal autonomic tone or hyperresponsiveness of the sinus node to acetylcholine, have been proposed as the underlying pathophysiology of the syndrome (Mandel et al., 1972; Ferrer, 1973). In any case, other contributing factors must be sought, since autonomic blockade does not always abolish these long pauses (Narula et al., 1972; Gupta et al., 1974; Jordan et al., 1978). The present results indicate that marked post-overdrive suppression with long pauses of over several seconds can occur in sinus node pacemaker cells when active ion transport or metabolic activity of the cells is depressed. Thus, the long pauses following overdrive or tachycardia observed in patients with sick sinus syndrome may be ascribed in part to metabolic dysfunction of sinus node pacemaker cells.

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doi: 10.1161/01.RES.46.1.90

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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