Properties of Oscillatory Afterpotentials in Young Embryonic Chick Hearts

Michio Kojima and Nick Sperelakis

From the Department of Physiology, University of Cincinnati College of Medicine, Cincinnati, Ohio

SUMMARY. Oscillatory afterpotentials in cardiac tissues are believed to result from an activation of calcium-operated cation channels of mixed selectivity (sodium, potassium), or perturbation of an electrogenic sodium-calcium exchange carrier, by an oscillatory release of calcium from sarcoplasmic reticulum. In the present experiments, the presence and properties of ouabain-induced oscillatory afterpotentials were examined in young (3-day-old) embryonic chick hearts, both fresh and organ cultured for 6–14 days. The hearts did not differentiate in organ culture, and the cells retained slowly rising spontaneous action potentials. To induce the oscillatory afterpotentials, automaticity was suppressed by elevating extracellular K+ ion concentration from 4 mM to 6 mM, and the preparations were electrically stimulated at a rate of 0.5 Hz. Stable oscillatory afterpotentials were induced with 1.3–7.5 μM ouabain. The oscillatory afterpotential amplitude was increased when the stimulation rate was increased to 3 and 4 Hz. The oscillatory afterpotentials were potentiated when extracellular Ca++ ion concentration was increased to 3.6 mM or by the addition of barium (0.1 mM). Low extracellular Na+ ion concentration (40–121 mM), strontium (4 mM), magnesium (2–4 mM), and manganese (1–4 mM) significantly depressed the oscillatory afterpotentials. These properties of the oscillatory afterpotentials are similar to those described for adult mammalian ventricular muscles and Purkinje fibers. Our results suggest that young embryonic chick hearts (which lack fast sodium channels and, instead, have slow sodium channels) possess the calcium-operated mixed cation channels at a very early developmental stage if the oscillatory afterpotentials result from the activation of the mixed cation channels by intracellular calcium. (Circ Res 55: 497–503, 1984)

OSCILLATORY afterpotentials (OAP) or transient inward current (Iₜₐ) in cardiac tissues develop in the presence of digitalis (Cranefield, 1977; Ferrier, 1977), caffeine (Clusin, 1983), grayanotoxin-III (Brown et al., 1981), in K+-free or high extracellular Ca++ ion concentration [Ca++], solutions (Eisner and Lederer, 1979; Hiraoaka et al., 1979; Vassalle and Mugelli, 1981; Kass and Tsien, 1982), and following depolarizing voltage clamp steps (Vassalle and Mugelli, 1981; Mugelli, 1982; Lipsius and Gibbons, 1982). Digitalis-induced OAP have been reported to occur in Purkinje fibers (Rosen et al., 1973; Ferrier et al., 1973; Lederer and Tsien, 1976; Ishikawa and Vassalle, 1982), ventricular muscles (Ferrier, 1976; Karagueuzian and Katzung, 1981, 1982), atrial muscles (Hashimoto and Moe, 1973; Hordof et al., 1978), cultured heart cells (Goshima, 1977), and isolated single ventricular cells (Matsuda et al., 1982). Delayed afterdepolarizations (DAD) were also observed in cultured chick embryonic heart cells in the absence of cardiac glycoside, i.e., they occurred naturally (Sperelakis, 1972).

Recent voltage clamp experiments have indicated that the OAP are generated by an Iₜₐ which is distinguishable from the normal pacemaker current. It was suggested that the OAP and Iₜₐ result from an activation of Ca++-operated mixed cation (Na+,K+)-channels, or perturbation of an electrogenic Na-Ca exchange carrier, by an oscillatory release of Ca++ from intracellular stores (sarcoplasmic reticulum (SR)) which are overloaded with Ca++ (Lederer and Tsien, 1976; Kass et al., 1978a, 1978b; Karagueuzian and Katzung, 1982).

The ionic channels possessed by chick hearts undergo sequential changes during development (Sperelakis, 1972, 1980; Sperelakis and Pappano, 1983). Young embryonic (2- to 3-day-old) hearts either lack or have very few fast Na+ channels and, instead, possess primarily tetrodotoxin (TTX)-insensitive slow Na+ channels, whereas older (16- to 21-day-old) embryonic hearts have a large number of TTX-sensitive fast Na+ channels and few or no slow Na+ channels (but a full complement of slow Ca++-Na+ channels). Therefore, the young embryonic hearts have slowly rising action potentials (AP) dependent on the slow Na+ channels, whereas the old embryonic hearts have fast-rising AP dependent on the fast Na+ channels.

The purpose of the present experiments was to examine whether ouabain induces OAP in the young embryonic hearts (whose channel population is different from the old embryonic hearts), and, if so, to determine their characteristics. It was found that ouabain induces OAP in the heart at an early developmental stage.
Methods

Preparations and Solutions

Young (3-day-old) embryonic chick hearts, fresh or organ cultured, and old (16-day-old) fresh hearts were used. The organ culture techniques were described previously (Sperelakis and Shigenobu, 1974; Renaud and Sperelakis, 1976). Fertilized chicken eggs were incubated at 37°C, and the ventricular portion of the heart was removed from 3-day-old embryos. The hearts were incubated in culture medium (medium 199 with Earle’s salts, GIBCO Lab.) for 3–14 days.

Each culture preparation was placed in an experimental chamber (0.5-ml volume) and superfused at a flow rate of 2.5 ml/min with a peristaltic pump. The control Tyrode’s solution had the following composition (in mM): 137 NaCl, 4.0 KCl, 0.6 CaCl₂, 1.1 MgCl₂, 24 NaHCO₃, 0.42 NaH₂PO₄, and 5.5 glucose. For the low extracellular Na⁺ concentration [Na⁺]₀-Tyrode’s solutions, NaCl was replaced with sucrose to maintain osmolarity. The divalent cations used (Ba++, Sr ++, Mg ++, and Mn ++) were in the form of the chloride salt. The superfusion medium was gassed with 95% O₂-5% CO₂ (pH of 7.3–7.4), and the temperature was maintained at 35 ± 0.5°C.

Action Potential Recording

Intracellular recording was carried out using conventional 3 M KCl-filled glass microelectrodes (resistance of 20–40 MΩ). The reversible half-cells were Ag-AgCl wires; the bath was grounded through an agar-Ringer solution bridge. The microelectrode was connected to a preamplifier (Dagan, model 8500) with a high input-impedance electrometer and negative capacitance compensation. The first time derivative of the AP upstroke (dV/dt) was obtained with an RC differentiator (time constant of 0.5 or 1 msec). Both the AP and dV/dt were displayed on a dual-beam oscilloscope (Tektronix, model 565) and photographed. For driven AP, the heart was stimulated (pulses of 1 msec duration) at a rate of 0.5 Hz by field stimulation using two platinum electrodes.

Experimental Protocol

After stable spontaneous AP were obtained (usually over a 10-minute period), the following procedure was followed. (1) extracellular K⁺ ion concentration [K⁺]₀ was increased from 4 to 6 mM to stop or reduce the rate of spontaneous AP (<0.5 Hz). (2) [Ca²⁺]₀, was increased from 0.6 to 2.4 mM. (3) The preparation was stimulated at the rate of 0.5 Hz in the continuous presence of 3.8 μM ouabain to induce OAP. (4) The concentrations of [Ca²⁺]₀ and ouabain were readjusted to maintain stable and marked OAP. The optimum concentration of [Ca²⁺]₀ was between 1.2 and 3.6 mM, and that of ouabain was between 1.3 and 7.5 μM. Well-developed OAP were usually obtained within 30 minutes after the addition of ouabain. The amplitude of the OAP was measured from the maximum diastolic potential, immediately preceding the OAP, to the peak of the first OAP. The amplitude of the OAP remained almost constant for at least 1 hour thereafter, and experiments were carried out during this period.

Statistical Analysis

All data are presented as mean ± SEM. Differences from the control value were tested by one-way analysis of variance. When the analysis of variance was significant (P < 0.05), the following statistical evaluations were made: comparisons involving two groups were performed by Student’s paired t-test (two-tailed), whereas comparisons involving more than two groups were by the Bonferroni’s method (Wallenstein et al., 1980). Calculations and statistical analyses were done with the aid of a computer (ACOS-800/Library 6, NEC). A probability of <0.05 was considered statistically significant.

Results

Ouabain Induction of OAP in 16-Day-Old Embryonic Hearts

Ouabain induced OAP in 16-day-old embryonic hearts. The OAP were observed as one or more low-amplitude potentials (<20 mV) during the diastolic interval, if this interval was long enough. A typical experiment is shown in Figure 1. The hearts were stimulated at a rate of 0.5 Hz (in 4 mM [K⁺]₀-Tyrode’s solution) (Fig. 1A). The addition of ouabain (5 μM) induced stable OAP which were maintained for more than 1 hour (Fig. 1B). The OAP amplitude was potentiated at higher frequencies of drive (Fig. 1, C and D). Washout of the drug reversed the ouabain-induced effects within 30 minutes (Fig. 1E).

Ouabain Induction of OAP in 3-Day-Old Embryonic Hearts

Fresh Hearts

Three-day-old fresh (noncultured) hearts had regular spontaneous APs. In 4 mM and 6 mM [K⁺]₀, ouabain (1.3–7.5 μM) caused a slight decrease of the maximal diastolic potential. At ≥2.5 μM concentrations of ouabain, small OAP appeared transiently in two out of five experiments in 4 mM [K⁺]₀, and in seven out of 16 experiments in 6 mM [K⁺]₀. However, the OAP in both [K⁺]₀ levels could not be sustained due to the development of faster firing or low-amplitude oscillations (not illustrated). Therefore, because of the relative instability of the OAP in the fresh hearts, characterization of the OAP was done primarily in the following organ-cultured hearts.

Organ-Cultured Hearts

Three-day-old hearts placed into organ culture (for up to 14 days) retain slow Na⁺ channels and do not gain fast Na⁺ channels (Sperelakis and Shigenobu, 1974). In the present experiments, the plateau phase of the slow AP, which is dependent on [Ca²⁺]₀ and depressed by Mn⁺⁺ (a specific slow Ca²⁺ channel blocker), became longer with increasing period of culture. This prolongation of AP duration could reflect an increase in the number of the slow channels.

In 4 mM [K⁺]₀, the organ-cultured hearts had spontaneous activity. Addition of ouabain (2.5–5.0 μM) induced OAP transiently in 11 out of 28 experiments (not illustrated).

In 6 mM [K⁺]₀, the spontaneous activity was either
Kojima and Sperelakis/Delayed Afterpolarizations in Young Embryonic Hearts

FRESH 16-DAY-OLD EMBRYONIC HEARTS

Control

Ouabain 5.0 μM
20 min 0.5 Hz

3 Hz

5 Hz

Washout

20 min

400 ms

FIGURE 1. Ouabain induction of oscillatory afterpotentials (OAP) in fresh 16-day-old embryonic chick hearts. In each panel, the upper, middle, and lower traces represent zero potential, action potential (AP), and dV/dt, respectively. Panel A: control AP of 16-day-old embryonic heart. Panel B: ouabain (5.0 μM) induced stable OAP. Panels C–D: about 20 pulses at 3 Hz (C) and 5 Hz (D) induced larger OAP than did a stimulation at 0.5 Hz (B). Panel E: washout of the ouabain restored the AP to the control pattern. Solutions contained 4 mM [K+]o and 2.4 mM [Ca++]o.

greatly reduced (<0.5 Hz) or abolished. Electrical stimulation (at 0.5 Hz) was applied in the presence of different concentrations of ouabain. When the culture period was longer than 5 days, ouabain (3.8 μM) was able to induce stable OAP in every preparation within 30 minutes (Fig. 2C). Under these conditions, stable ouabain-induced OAP were maintained for at least 1 hour thereafter. Further increase in ouabain (5.0 μM) first produced larger OAP (Fig. 2D), and then induced irregular spontaneous firing (Fig. 2E). The following studies were carried out in the conditions giving the stable OAP.

Properties of Ouabain-induced OAP in the Organ-Cultured 3-Day-Old Embryonic Hearts

Frequency Dependence

Because OAP amplitude depends on stimulation rate in adult heart tissues, the effects of frequency changes on the ouabain-induced OAP were examined in the organ-cultured hearts. The results from five experiments are summarized in Table 1. There was no significant change in the amplitude of the OAP between stimulation rates of 0.1 and 2 Hz (P > 0.05). Increasing the stimulation rate from 0.1 Hz to 3 and 4 Hz increased in the amplitude of the OAP from 5.8 ± 0.8 mV to 7.6 ± 1.0 mV and 11.4 ± 1.3 mV, respectively (P < 0.05). At 6 Hz, there was a slight, but insignificant, increase in the amplitude of the OAP (6.1 ± 0.7 mV, P > 0.05). That is, increasing the rate from 0.1 Hz to 6 Hz showed a biphasic change in the OAP amplitude.

Effects of [Na+]o

Effects of low [Na+]o on the ouabain-induced OAP were examined to determine the importance of Na+ for the development of the OAP. When the normal [Na+]o (161 mM) was reduced, the amplitude of the OAP was reduced (Fig. 3, upper row). Reductions

ORGAN-CULTURED 3-DAY-OLD EMBRYONIC HEARTS

Culture Period of 11 Days

Control

Ouabain 2.5 μM
15 min

3.8 μM
30 min

5.0 μM
10 min

5.0 μM
13 min

FIGURE 2. Ouabain induction of OAP in organ-cultured 3-day-old embryonic chick hearts. Panel A: control AP of the heart cultured for 11 days. Panels B–C: ouabain did not induce the OAP at 2.5 μM (panel B), but induced stable OAP at 3.8 μM (panel C). Panels D–E: ouabain, 5.0 μM further increased the amplitude of the OAP (panel D), 10 minutes and then induced a triggered-activity (panel E, 13 minutes). The solution contained 6 mM [K+]o and 2.4 mM [Ca++]o. The preparation was stimulated at a rate of 0.5 Hz.
TABLE 1
Dependence of the Amplitude of the OAP on the Frequency of Stimulation in Organ-Cultured 3-Day-Old Embryonic Chick Hearts

<table>
<thead>
<tr>
<th>Frequency of stimulation (Hz)</th>
<th>No. of experiments</th>
<th>OAP amplitude (mV)</th>
<th>Absolute value</th>
<th>Difference value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>5</td>
<td>5.8 ± 0.8</td>
<td>5.8 ± 0.8</td>
<td>-0.0 ± 0.3</td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
<td>5.8 ± 0.8</td>
<td>5.8 ± 0.8</td>
<td>0.0 ± 0.3</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>5.2 ± 0.7</td>
<td>5.2 ± 0.7</td>
<td>-0.6 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>6.8 ± 0.8</td>
<td>6.8 ± 0.8</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>7.6 ± 1.0</td>
<td>7.6 ± 1.0</td>
<td>1.8 ± 0.5†</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>11.4 ± 1.3</td>
<td>11.4 ± 1.3</td>
<td>5.6 ± 0.9†</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>6.1 ± 0.7</td>
<td>6.1 ± 0.7</td>
<td>0.3 ± 0.7</td>
</tr>
</tbody>
</table>

Data given as mean ± SEM. The OAP amplitudes were measured after a train of 20 pulses were applied at each frequency (i.e., after equilibration was attained for each frequency).

* Difference from the value of 0.1 Hz.
† Statistically significant difference from the value at 0.1 Hz at \( P < 0.05 \).

Effects of Divalent Cations

Since \( Ca^{++} \) is necessary for the genesis of the OAP in adult hearts, it was determined whether the OAP in the 3-day-old organ-cultured hearts were dependent on \([Ca^{++}]_o\). Figure 3 (lower row) shows one such experiment. As can be seen, increases in \([Ca^{++}]_o\) from 1.2 mM to 1.8, 2.4, 3.0, and 3.6 mM increased the amplitude of the OAP dose-dependently from \(0.3 \pm 0.3 \text{ mV} \) \((n = 5)\) to \(4.9 \pm 1.4 \text{ mV} \) \((n = 5)\), \(5.6 \pm 1.2 \text{ mV} \) \((n = 4)\), \(8.1 \pm 1.8 \text{ mV} \) \((n = 4)\), and \(11.3 \pm 2.4 \text{ mV} \) \((n = 4)\), respectively. A plot of OAP amplitude vs. \( \log [Ca^{++}]_o \) gave a slope of 20 mV/decade (Fig. 4). The \([Ca^{++}]_o\)-induced changes were reversed within 30 minutes when the \([Ca^{++}]_o\) was returned to 1.2 mM within 30 minutes (Fig. 3J).

Since it was reported that \( Ba^{++} \) induces OAP, and \( Sr^{++} \), \( Mg^{++} \), and \( Mn^{++} \) reduce or abolish OAP and \( I_o \) in adult heart tissues, the effects of these cations were examined on the OAP in the 3-day-old organ-cultured hearts. Table 2 summarizes these data. A low concentration of \( Ba^{++} \) (0.1 mM) first increased the OAP amplitude from \(6.3 \pm 2.3 \text{ mV} \) \((n = 4, P < 0.05)\), and then induced irregular spontaneous activity (not illustrated). \( Sr^{++} \), \( Mg^{++} \), and low \([Na^+]_o\) were reversible within 30 minutes when the \([Na^+]_o\) was returned to control (Fig. 3E).

FIGURE 3. Effects of changes in \([Na^+]_o\) (panels A–E) and \([Ca^{++}]_o\) (panels F–J) on ouabain-induced OAP in organ-cultured 3-day-old embryonic chick hearts. Panels A–3: \([Na^+]_o\) was reduced from 100% (control A) to 75% (panel B), 50% (panel C), and 25% (panel D) of the control value (161 mM), and returned to 100% (panel E). Note the amplitudes of the OAP, as well as the slow AP, decreased as \([Na^+]_o\) was lowered. The solutions contained 3.8 \( \mu \text{M} \) ouabain, 6 mM \([K^+]_o\), and 2.4 mM \([Ca^{++}]_o\); the culture period was 8 days. Panels F–J: \([Ca^{++}]_o\) was increased from 1.2 mM (panel F) to 1.8 mM (panel G), 2.4 mM (panel H), and 3.0 mM (panel I), and returned to 1.2 mM (panel J). Note that amplitude of the OAP increased as \([Ca^{++}]_o\) was raised. The solution contained 3.8 \( \mu \text{M} \) ouabain and 6 mM \([K^+]_o\); the culture period was 7 days. Each preparation was stimulated at a rate of 0.5 Hz.
and Mn++ (1-4 mM) had dose-dependent depressant effects on the OAP. At 4 mM, Sr++, Mg++, and Mn++ significantly decreased the amplitude of the OAP from 6.1 ± 1.5 mV to 1.9 ± 0.9 mV \((n = 5, P < 0.05)\), from 5.4 ± 1.1 mV to 1.8 ± 0.3 mV \((n = 4, P < 0.05)\), and from 9.4 ± 1.7 mV to 2.3 ± 0.6 mV \((n = 4, P < 0.05)\), respectively. The cation-induced changes were reversed within 30 minutes when the preparations were superfused with ouabain-containing Tyrode's solution without these ions.

### Discussion

**Mechanism for Digitalis-Induced OAP**

Several studies have attempted to determine the mechanisms responsible for digitalis-induced OAP and \(I_h\) (Kass et al., 1978a, 1978b; Vassalle and Mugelli, 1981; Matsuda et al., 1982; Karagueuzian and Katzung, 1982). When the Na-K pump is inhibited by digitalis, sodium ions accumulate within the cell, and therefore an increase in \([Ca^{++}]_i\) results from a reduction in \([Ca^{++}]_o\) efflux via the Na-Ca exchange. The increased \([Ca^{++}]_i\) would cause overloading of the SR with \([Ca^{++}]_i\). In addition, the increased \([Ca^{++}]_o\) may cause an oscillatory release of \([Ca^{++}]_i\) from the intracellular SR stores and may induce OAP. Two possible mechanisms have been proposed for genesis of \(I_h\) or OAP (Kass et al., 1978b; Tsien et al., 1979): (1) Mixed (Na+,K+) cation channels, somewhat analogous to acetylcholine-activated channels at the motor end-plate, are activated by the oscillatory increase in \([Ca^{++}]_j\). (2) The OAP may reflect an electrogenic Na-Ca exchange carrier modulated by the oscillatory increase in \([Ca^{++}]_i\).

**Ouabain-Induced OAP in Young Embryonic Chick Hearts**

The present results show that ouabain transiently induced OAP in fresh 3-day-old embryonic hearts, but the OAP were more consistent and stable in the organ-cultured 3-day-old embryonic hearts (especially in the hearts cultured for more than 5 days). Since the slow \([Ca^{++}]_o\)-Na+ channels increase during organ culture (unpublished observations), a greater \(I_n\) through more slow channels is expected to cause a higher \([Ca^{++}]_i\), and thereby a greater \(I_h\), which is responsible for stable OAP. It is also possible that the number of slow \([Ca^{++}]_o\)-Na+ channels and of \([Ca^{++}]_o\)-operated mixed cation channels increase in parallel.

### Table 2

<table>
<thead>
<tr>
<th>Divalent Cation Species</th>
<th>Concentration (mM)</th>
<th>No. of experiments</th>
<th>OAP Amplitude (mV) Absolute Value Difference Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ba++</td>
<td>Control</td>
<td>4</td>
<td>6.3 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Sr++</td>
<td>Control</td>
<td>5</td>
<td>6.1 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Mg++</td>
<td>Control</td>
<td>4</td>
<td>5.4 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Mn++</td>
<td>Control</td>
<td>4</td>
<td>9.4 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Data given as mean ± SEM.

* Difference from the control value.
† Statistically significant difference from the control value at \(P < 0.05\).
However, which is the correct explanation for the larger OAP in the organ-cultured hearts is not known. The fact that ouabain-induced OAP in 3-day-old embryonic hearts suggests that these young hearts have Ca++-operated mixed cation channels for the OAP, as do adult cardiac hearts, if the OAP result from the activation of the mixed cation channels by intracellular Ca++. The fact that an increase in [K+]o from 4 to 6 mM allowed the induction of more stable OAP by ouabain is in agreement with reports in the literature. Rosen et al. (1973) reported that the ouabain-induced OAP in 4–5 mM [K+]o were a little greater than those in 2.5 mM [K+]o in canine Purkinje fibers. In contrast, the acetylstrophanthidin-induced OAP in canine Purkinje fibers were increased in low [K+]o (2 mM) and abolished in high [K+]o (6–10 mM) (Ferrier and Moe, 1973; Hashimoto and Moe, 1973). Kass et al. (1978b) reported that a reduction of [K+]o from 4 to 1 mM caused no significant alteration in strophanthidin-induced in calf Purkinje fibers.

Frequency Dependence of Ouabain-Induced OAP

The observed increase in amplitude of the ouabain-induced OAP by increasing the stimulation rate, especially between 2 and 4 Hz, is in agreement with the literature (Ferrier et al., 1973; Ferrier, 1976; Rosen and Danilo, 1980; Karagueuzian and Katzung, 1981). However, the optimum cycle length to produce the maximum amplitude of the OAP was different in the various studies. Rosen and Danilo (1980) (in canine Purkinje fibers) and Karagueuzian and Katzung (1981) (in guinea pig papillary muscles) found, as we did, that the amplitude of the OAP increased with decreasing the cycle length from 1000 msec to 200 msec. In contrast, Ferrier et al. (1973) and Ferrier (1976) (in canine Purkinje fibers) reported that the amplitude of the OAP increased as the cycle length was decreased from 1000 msec to 600 msec, but further decrease of the cycle length decreased the amplitude. In the present studies, the maximum amplitude of the OAP was observed when the cycle length became equal to the coupling interval (the interval from the upstroke of the last driven AP to the peak of the first OAP). This suggests that the maximum amplitude of the OAP is obtained when the timing of the AP coincides with the cycle of oscillatory release of Ca++ from the SR (Orchard et al., 1983).

Effects of Na+ and Ca++ on Ouabain-Induced OAP

The present results show that lowering [Na+]o caused decreases in the amplitude of the ouabain-induced OAP. Low [Na+]o was reported to depress the digitalis-induced OAP or Ia in canine or calf Purkinje fibers (Lin and Vassalle, 1978; Kass et al., 1978b), in guinea pig or ferret papillary muscles (Karagueuzian and Katzung, 1982; Arlock and Katzung, 1982), and in canine papillary or trabecular muscles (Hiraoka et al., 1979). A reduction of [Na+]o decreases the driving force for Ia, which would favor a decrease in OAP amplitude. A reduction of [Na+]o could reduce Ca++ efflux via the Na-Ca exchange mechanism or increase the Ca++ influx by changing the balance of Na+-Ca++ competition at the slow Ca++-Na+ channels; this would result in an increase in [Ca++], thus favoring an increase in the OAP. Since OAP amplitude was decreased in low [Na+]o, the former factor must have predominated.

The present studies show that the ouabain-induced OAP are dependent on [Ca++]o. The higher the [Ca++]o, the larger the OAP. Similar [Ca++]o-dependence of OAP or Ia has been reported in canine and calf Purkinje fibers (Ferrier and Moe, 1973; Rosen et al., 1974; Kass et al., 1978a), in ferret papillary muscles (Karagueuzian and Katzung, 1982), and in isolated single ventricular cells (Matsuda et al., 1982). It is possible that Ia channels carry some of the Ia, as suggested by the unexpected large slope (of 20 mV/decade) found for the relationship between OAP amplitude and log [Ca++]o in comparison with the much lower slope for Na+ (only 9 mV/decade) (Fig. 4). Alternatively, the Ia channels may also allow Ca++ to pass through.

Effects of Other Divalent Cations on Ouabain-Induced OAP

In the present experiments, Ba++ increased the ouabain-induced OAP, whereas Sr++, Mg++, and Mn++ reduced or abolished the OAP. These results are consistent with previous results in various adult heart tissues. For example, Ba++ was reported to induce OAP in dog ventricular muscles and sheep Purkinje fibers (Reid and Hecht, 1967; Mugelli et al., 1983). Mg++ and Mn++ depressed the digitalis-induced OAP or Ia in canine and calf Purkinje fibers (Ferrier and Moe, 1973; Rosen et al., 1974; Kass et al., 1978a). Since the divalent ion selectivity of cardiac slow Ca++-Na+ channels is: Ba++ > Sr++ > Ca++ >> Mg++ (Sperelakis and Lehmkuhl, 1966; Kohlhardt et al., 1973; McDonald, 1982), these ions enter the cells through the slow channels. However, the intracellular effects of these ions on the Ca++-activated mixed (Na+,K+) cation channels, Ca++ uptake by the SR, and Ca++-induced release of Ca++ release from the SR are not known. Thus, we do not know how these ions affect [Ca++], and, thereby, the OAP. However, the suppression of the OAP by Mn++, a blocker of slow channels, may be a secondary effect of decreased Ia and reduced [Ca++]o.

Conclusions

Ouabain induced stable OAP in organ-cultured 3-day-old embryonic hearts, but induced OAP transiently in the fresh hearts. The characteristics of the OAP, i.e., the frequency- and [Na+]o-dependence and the responses to divalent cations, are quite
similar to those previously described in adult cardiac tissues. If the OAP result from activation of the mixed cation channels by intracellular Ca++, these results suggest that young embryonic chick hearts have these channels at a very early developmental stage.

This research was supported by Grants HL-18711 and HL-31942 from the National Institutes of Health.

Address for reprints: Dr. Nick Sperelakis, Department of Physiology/mL #576, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Received December 16, 1983; accepted for publication July 20, 1984.

References

Ferrier GR (1977) Digitalis arrhythmias: Role of oscillatory afterpotentials. Prog Cardiovasc Dis 19:459-474
Ferrier GR, Moe GK (1973) Effect of calcium on acetylstrophanthin-induced transient depolarizations in canine Purkinje tissue. Circ Res 33:508-515
Ferrier GR, Saunders JH, Mendez C (1973) A cellular mechanism for the generation of ventricular arrhythmias by acetyls

Kohlhardt M, Haastert HP, Krause H (1973) Evidence of non-specificity of the Ca channel in mammalian myocardial fibre membranes. Substitution of Ca by Sr, Ba or Mg as charge carriers. Pflugers Arch 342:125-136
Lederer WJ, Tsien RW (1976) Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibers. J Physiol (Lond) 263:73-100

INDEX TERMS: Oscillatory afterpotentials • Delayed afterdepolarizations • Calcium-operated cation channels • Cardiac glyc- coside action • Differentiation of myocardial cells • Developmental electrophysiology
Properties of oscillatory afterpotentials in young embryonic chick hearts.

M Kojima and N Sperelakis

doi: 10.1161/01.RES.55.4.497

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1984 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/55/4/497

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org/subscriptions/