Anesthetics and Automaticity in Latent Pacemaker Fibers

III. Effects of Halothane and Ouabain on Automaticity of the SA Node and Subsidiary Atrial Pacemakers in the Canine Heart

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Atrial tachyarrhythmias are a common manifestation of digitalis toxicity. Such arrhythmias could be due to enhanced automaticity of subsidiary atrial pacemakers (SAP) compared to the sinoatrial (SA) node. Halothane is known to oppose digitalis-induced ventricular arrhythmias. Its effect on digitalis-caused atrial arrhythmias is unknown. Therefore, we tested two hypotheses, as follows. First, increasing ouabain concentrations would enhance automaticity of SAP compared to the SA node and that such enhanced automaticity could explain digitalis-caused atrial tachyarrhythmias. Second, halothane would oppose such enhanced automaticity of SAP, thereby opposing digitalis-caused atrial tachyarrhythmias. A canine right atrial preparation was perfused via the SA node artery with Krebs’ solution (36.0 ± 0.5°C) equilibrated with 97% oxygen–3% carbon dioxide. Four bipolar extracellular electrodes recorded the site of earliest activation (SEA), which in this preparation could be the SA node or increasingly remote sites of SAP approximately 1, 2, and 3 cm distal to the SA node along the sulcus terminalis. Pacemaker shifts to SAP during exposure to drugs were scored for magnitude of shift as 1, 2, or 3 depending on which SAP site was the SEA. Magnitude scores were summed for each test condition and normalized by dividing the total number of preparations tested. Preparations (N = 48) were exposed to 1 or 2% halothane (perfusate concentrations of 0.51 ± 0.01 or 0.79 ± 0.03 ml/ml, respectively) and/or to low- or mid-therapeutic (2.5 or 5 × 10^-4 M) or borderline toxic ouabain (1 × 10^-3 M). Normalized magnitude scores were not significantly different from zero (control value) with any halothane or ouabain concentration alone. The normalized magnitude score for 1% halothane with 1 × 10^-7 M ouabain (0.56) was borderline significant (P = 0.055), and scores for 2% halothane with 5 × 10^-4 M (0.47) or 1 × 10^-3 M ouabain (1.16) were significantly different from zero (P < 0.05). Regardless of pacemaker location, spontaneous heart rate tended to be increased by ouabain and slowed by halothane alone; this tendency was most pronounced for 1 × 10^-7 M ouabain or 2% halothane. In preparations with pacemaker shifts, spontaneous rate was not different from control. Finally, total atrial arrest was observed in six preparations exposed to 1 or 2% halothane with 1 × 10^-7 M ouabain. It is concluded that in the perfused canine right atrial preparation, increasing ouabain does not sufficiently enhance automaticity of SAP or SA node to account for digitalis-caused atrial tachyarrhythmias. Further, borderline toxic ouabain with halothane favors pacemaker shifts from the SA node to sites of SAP and in some preparations may cause atrial electrical quiescence. (Key words: Anesthetics, volatile: halothane. Animal: dog. Heart: arrhythmias; electrophysiology; normal automaticity; sinus node; subsidiary atrial pacemakers; cardiac glycosides, ouabain.)

DISORDERS OF SINUS RHYTHM AND ATRIAL ARRYTHMIAS ARE COMMON MANIFESTATIONS OF DIGITALIS TOXICITY.1 THE MECHANISM FOR SUCH RHYTHM DISTURBANCES MUST INVOLVE A COMPLEX INTERPLAY OF INDIRECT (NEURALLY MEDIATED) AND DIRECT ELECTROPHYSIOLOGIC ACTIONS OF DIGITALIS.1 FOR EXAMPLE, STUDIES IN CHRONICALLY INSTRUMENTED DOGS INDICATE THAT OUABAIN SLOWS THE RATE OF SINOATRIAL (SA) DISCHARGE.2 IT ALSO PRODUCES SA ARREST AND BLOCK, AND IN SOME INSTANCES, PRODUCES SHIFTS OF THE PACEMAKER AWAY FROM THE SA NODE.2 SINCE THESE EFFECTS WERE SIMILAR TO THOSE OF ACYCTOHELME OR VAGAL STIMULATION AND WERE ABOLISHED BY ATROPINE, IT WAS SUGGESTED THAT OUABAIN'S EFFECT WAS VAGALLY MEDITATED. IN CONTRAST TO IN VIVO STUDIES, IN THE ISOLATED HEART OUABAIN PRODUCED ACCELERATION OF THE SA NODE DISCHARGE RATE WITH PERIODS OF BEAT-TO-BEAT ALTERATION IN THE ATRIAL INTERVALS.3 THIS WAS FOLLOWED IN TURN BY A RAPID RATE AND RHYTHM, COMPARABLE TO THAT WHICH MIGHT BE OBSERVED WITH PAROXYSMAL ATRIAL TACHYCARDIA.3 IN THOSE EXPERIMENTS, WHICH USED ATRIAL ACTIVATION MAPPING, PACEMAKER ACCELERATION WAS ACCOMPANIED BY A SHIFT IN THE DOMINANT PACEMAKER SITE (SA NODE) TOWARD THE SA BORDER.3 DESPITE DIFFERENCES IN THE EFFECT OF OUABAIN ON SA NODE RATE BETWEEN IN VIVO AND IN VITRO STUDIES, COMMON TO BOTH WAS THE OBSERVATION THAT OUABAIN TENDED TO PRODUCE PACEMAKER SHIFTS AWAY FROM THE SA NODE.3,5 THIS SUGGESTS THE POSSIBILITY THAT ENHANCED AUTOMATICITY IN SUBSIDIARY ATRIAL PACEMAKERS (SAP) MAY BE INVOLVED IN THE GENESIS OF ATRIAL TACHYARRHYTHMIAS DUE TO DIGITALIS TOXICITY, ALTHOUGH WE CANNOT OVERLOOK THE POSSIBLE INVOLVEMENT OF DELAYED AFTERPOLARIZATIONS WITH TRIGGERED ACTIVITY, OR REENTRY OF EXCITATION.1

In the canine heart, SAP are located inferior to the classic SA node region4 along the sulcus terminalis but within the distribution of the SA node artery.5-7 Other reported sites of subsidiary pacemaker activity (dog or rabbit) within the atria include the coronary sinus,8 Bachmann's bundle,5,6,9 atrial plateau fibers,10,11 and the atrioventricular valve leaflets.12 None of these additional potential sites of atrial pacemaker activity has been so

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thoroughly examined as SAP along the sulcus terminalis.\textsuperscript{5,7,13–22} Presumably, SAP provide a fail-safe mechanism in case the SA node defaults; they also may explain a multicentric atrial pacemaker complex.\textsuperscript{23–25} SAP also may function as a source of abnormal rhythmic activity.\textsuperscript{22}

To our knowledge, digitalis effects on automaticity of SAP along the sulcus terminalis have not been reported, although reports cited above\textsuperscript{18,19} suggest that automaticity of these pacemakers might be enhanced relative to that of the SA node by toxic levels of digitalis.

Halothane directly depresses SA node automaticity,\textsuperscript{26,27} although such depression may not be clinically apparent due to the effects of other drugs or autonomic compensatory mechanisms.\textsuperscript{27,28}

Although the direct effect of halothane on SAP automaticity is not known, in a perfused canine right atrial preparation halothane alone does not augment automaticity of SAP relative to the SA node.\textsuperscript{29}

With halothane and epinephrine or norepinephrine, however, SAP automaticity is augmented, as demonstrated by pacemaker shifts from the SA node to the above-described SAP sites along the sulcus terminalis.\textsuperscript{29}

The effect of halothane on digitalis-caused atrial arrhythmias is not known, although halothane does antagonize digitalis-caused ventricular arrhythmias.\textsuperscript{30–32} However, halothane cannot be presumed effective against digitalis-caused atrial arrhythmias, as has been suggested for atrial arrhythmias produced by hyperventilation (hypocapnic hypokalemia) in patients receiving digitalis.\textsuperscript{33}

The aforementioned considerations prompted testing, in a perfused canine right atrial preparation, of two hypotheses. First, increasing ouabain would sufficiently enhance automaticity of SAP compared to the SA node to account for digitalis-caused atrial tachyarrhythmias. Second, halothane might oppose ouabain-enhanced automaticity of SAP, thereby countering digitalis-caused atrial tachyarrhythmias.

**Materials and Methods**

This research was approved by the Medical College of Wisconsin Animal Care Committee and conforms with standards set forth in the National Institutes of Health Guide for Care and Use of Laboratory Animals.\textsuperscript{**}

Mongrel dogs of either sex (n = 48) weighing 10–22 kg were anesthetized with sodium pentobarbital (30 mg/kg intravenously). The heart with at least 2 cm of superior vena cava was quickly excised and immersed in cold, oxygenated (97% oxygen–3% carbon dioxide) Krebs' solu-

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\textsuperscript{**} Public Health Services, National Institutes of Health: Guide for Care and Use of Laboratory Animals. NIH Publication no. 85-23, revised 1985.
tion, as previously described. SA node artery cannulation and dissection to provide the right atrial preparation have also been described.

Four bipolar, extracellular recording electrodes (silver wire) were used to record the site of earliest activation (SEA), which could be the SEA node region or one of three increasingly remote sites of SAP (approximately 1, 2, and 3 cm) along the sulcus terminalis. Pacemaker shifts from the SA node to SAP produced by drugs were scored 1, 2, or 3 depending on which SAP site was the SEA. The magnitude of the shifts was then calculated for each experimental condition by adding scores for all preparations with pacemaker shifts from the SA node to produce a magnitude score. In turn, magnitude scores were normalized by dividing the magnitude score by the total number of preparations tested for a particular experimental condition.

Halothane (1 or 2%) was delivered from a calibrated vaporizer, and these concentrations were equivalent to measured perfusate concentrations of 0.51 ± 0.01 or 0.79 ± 0.03 mM, respectively, as determined by gas chromatography. Ouabain was added to the superfuse in concentrations of 2.5 × 10⁻⁸ M, 5 × 10⁻⁸ M, and 1 × 10⁻⁷ M. These ouabain concentrations are considered equivalent to low-therapeutic, mid-therapeutic, and borderline toxic, respectively.

Experiments lasted 4–5 h, during which time preparations were exposed to 1% (n = 14) or 2% (n = 34) halothane, and each of the three concentrations of ouabain (table 1). Five preparations were used to obtain time control data with ouabain alone (table 1). Data for the effects of halothane or ouabain on spontaneous rate of the preparations are shown as mean ± standard error of the mean. Statistical analysis was performed with ANOVA and paired and unpaired t tests as appropriate; results were considered significant if P was < 0.05.

### Table 2. Time Control Data for the Effect of Ouabain on Spontaneous Heart Rate

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Condition</th>
<th>Heart Rate (beats per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Cᵢ</td>
<td>70.0 ± 5.5</td>
</tr>
<tr>
<td>60</td>
<td>2.5 × 10⁻⁸ M ouabain</td>
<td>74.6 ± 5.5*</td>
</tr>
<tr>
<td>15</td>
<td>Cᵢ</td>
<td>74.2 ± 4.1</td>
</tr>
<tr>
<td>60</td>
<td>5 × 10⁻⁸ M ouabain</td>
<td>77.5 ± 5.8</td>
</tr>
<tr>
<td>15</td>
<td>Cᵢ</td>
<td>74.7 ± 5.3</td>
</tr>
<tr>
<td>60</td>
<td>1 × 10⁻⁷ M ouabain</td>
<td>87.6 ± 8.5</td>
</tr>
<tr>
<td>15</td>
<td>Cᵢ</td>
<td>75.6 ± 4.9</td>
</tr>
</tbody>
</table>

Data shown are mean ± SEM and were obtained from five preparations. Comparisons for each concentration of ouabain are with the respective control (Cᵢ, Cᵢ, or Cᵢ). Cᵢ = control value.

### Table 3. Effects of 1 or 2% Halothane and Ouabain on Spontaneous Heart Rate (beats per min)

<table>
<thead>
<tr>
<th>Condition</th>
<th>1% Halothane (n = 14)</th>
<th>2% Halothane (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cᵢ</td>
<td>91.9 ± 2.8</td>
<td>86.0 ± 2.2</td>
</tr>
<tr>
<td>H</td>
<td>85.6 ± 2.5*</td>
<td>75.5 ± 1.8*</td>
</tr>
<tr>
<td>Cᵢ</td>
<td>90.6 ± 2.7</td>
<td>82.9 ± 2.1</td>
</tr>
<tr>
<td>2.5 × 10⁻⁸ M ouabain</td>
<td>92.2 ± 2.7*</td>
<td>86.6 ± 2.3*</td>
</tr>
<tr>
<td>2.5 × 10⁻⁸ M ouabain + H</td>
<td>85.4 ± 2.2*</td>
<td>75.8 ± 2.0*†</td>
</tr>
<tr>
<td>Cᵢ</td>
<td>89.4 ± 2.7</td>
<td>83.6 ± 2.3</td>
</tr>
<tr>
<td>5 × 10⁻⁸ M ouabain</td>
<td>95.6 ± 2.6*</td>
<td>85.7 ± 2.5*</td>
</tr>
<tr>
<td>5 × 10⁻⁸ M ouabain + H</td>
<td>85.2 ± 2.4*†</td>
<td>76.1 ± 2.1*†</td>
</tr>
<tr>
<td>Cᵢ</td>
<td>91.5 ± 2.9</td>
<td>82.7 ± 2.3</td>
</tr>
<tr>
<td>1 × 10⁻⁷ M ouabain</td>
<td>98.2 ± 3.9*</td>
<td>91.2 ± 3.4*</td>
</tr>
<tr>
<td>1 × 10⁻⁷ M ouabain + H</td>
<td>103.9 ± 6.0*</td>
<td>91.2 ± 6.5</td>
</tr>
<tr>
<td>Cᵢ</td>
<td>103.5 ± 4.9*</td>
<td>81.9 ± 3.7</td>
</tr>
</tbody>
</table>

* P < 0.05 versus respective control. † P < 0.05 versus ouabain only. ‡ P < 0.05 versus Cᵢ.

### Results

#### Spontaneous Heart Rate

Time control data for the effect of ouabain on heart rate are shown in table 2. These indicate that only ouabain 2.5 × 10⁻⁸ M significantly increased heart rate as compared to control. Furthermore, heart rate after 4 h (75.6 ± 4.9 beats per min) was not significantly changed from that at the beginning of experiments (70.0 ± 3.5 beats per min) (table 2). In table 3 are values for heart rate after exposure to 1 or 2% halothane. Both concentrations of halothane decreased heart rate compared to control. All concentrations of ouabain increased heart rate compared to their respective controls. The addition of 1 or 2% halothane to 2.5 or 5 × 10⁻⁸ M ouabain decreased heart rate from values obtained with ouabain only. In contrast, 1 or 2% halothane did not oppose the increase in heart rate with 1 × 10⁻⁷ M ouabain.

### Pacemaker Shifts and Magnitude Scores

An isolated, perfused canine right atrial preparation with bipolar, extracellular recording electrode sites was illustrated in figure 1 of our companion report. In time control experiments with ouabain only, no shifts in the SEA from the SA node to SAP sites were observed for any of the concentrations of ouabain. Thus, magnitude scores and normalized magnitude scores were zero in all cases. Pacemaker shifts were observed, however, in experiments with halothane and ouabain. An example of one such shift is provided in figure 1. Pacemaker shifts from the SA node to sites of SAP are tabulated for experiments with 1% halothane in table 4 and for 2% halothane in table 5. In each of these tables are shown the number of preparations exhibiting pacemaker shifts per
number of preparations tested for each experimental condition, as well as summed magnitude scores (the sum of shifts to sites 1, 2, or 3) and normalized magnitude scores (the magnitude score divided by the number of preparations tested).

Neither 1% (table 4) nor 2% halothane (table 5) produced pacemaker shifts. Ouabain (2.5 or $5 \times 10^{-8}$ M) alone produced pacemaker shifts on occasion (tables 4 and 5), but normalized magnitude scores were not significantly different from control. In 4 of 26 preparations of the 2% halothane test group, ouabain $1 \times 10^{-7}$ M alone did produce pacemaker shifts (table 5). The corresponding normalized magnitude score for this particular test condition was borderline significant ($P = 0.059$). While there tended to be an increase in pacemaker shifts with 1% halothane and any concentration of ouabain (table 4), corresponding normalized magnitude scores were not significantly different from control (except for ouabain $1 \times 10^{-7}$; $P = 0.055$). With 2% halothane and $5 \times 10^{-8}$ or $1 \times 10^{-7}$ M ouabain (table 5), pacemaker shifts from the SA node to SAP sites were commonly observed and normalized magnitude scores were significantly different from control. Washout of halothane and ouabain effectively reversed pacemaker shifts with halothane and ouabain.

**Table 5. Pacemaker Shifts per Number of SA Node Preparations**

<table>
<thead>
<tr>
<th>Ouabain (M)</th>
<th>Shifts/SA Nodes (Magnitude Score)</th>
<th>Normalized Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% H</td>
<td>1% H</td>
</tr>
<tr>
<td>0</td>
<td>0/14</td>
<td>1/14</td>
</tr>
<tr>
<td>2.5 $\times 10^{-8}$</td>
<td>0/14</td>
<td>1/14</td>
</tr>
<tr>
<td>5 $\times 10^{-8}$</td>
<td>1/14</td>
<td>2/14</td>
</tr>
<tr>
<td>1 $\times 10^{-7}$</td>
<td>0/14</td>
<td>4/14</td>
</tr>
</tbody>
</table>

Shown are magnitude scores (parentheses) and normalized magnitude scores for experiments with 1% halothane (H) and ouabain. WO = washout.

$*P < 0.001$ versus 0 or $2.5 \times 10^{-8}$ M ouabain.

$\dagger P < 0.01$ versus 0% H or WO.
HALOTHANE, OUABAIN, AND ATRIAL PACEMAKER AUTOMATICITY

TABLE 6. Spontaneous Rate (beats per min) of Right Atrial Preparations with Pacemaker Shifts Compared to Those Without Shifts

<table>
<thead>
<tr>
<th>Condition</th>
<th>With</th>
<th>Without</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% H + 1 \times 10^{-7} M O</td>
<td>C_1: 95.7 ± 7.0 (4)</td>
<td>C_1: 91.1 ± 3.0 (10)</td>
</tr>
<tr>
<td></td>
<td>C_4: 94.1 ± 7.8 (4)</td>
<td>C_4: 90.5 ± 2.9 (10)</td>
</tr>
<tr>
<td></td>
<td>H + O: 98.3 ± 8.6 (4)</td>
<td>H + O: 106.4 ± 7.9 (10)**†</td>
</tr>
<tr>
<td></td>
<td>C_1: 83.2 ± 4.7 (10)</td>
<td>C_1: 87.2 ± 2.5 (24)</td>
</tr>
<tr>
<td></td>
<td>C_4: 80.3 ± 5.4 (10)</td>
<td>C_4: 84.5 ± 2.4 (24)</td>
</tr>
<tr>
<td></td>
<td>H + O: 74.0 ± 3.9 (10)**†</td>
<td>H + O: 77.0 ± 2.5 (24)**†</td>
</tr>
<tr>
<td></td>
<td>C_1: 86.5 ± 3.2 (16)</td>
<td>C_1: 85.6 ± 3.1 (9)</td>
</tr>
<tr>
<td></td>
<td>C_4: 84.5 ± 2.6 (16)</td>
<td>C_4: 82.9 ± 3.8 (9)</td>
</tr>
<tr>
<td></td>
<td>H + O: 97.1 ± 8.1 (18)</td>
<td>H + O: 81.5 ± 10.3 (9)</td>
</tr>
</tbody>
</table>

Shown is spontaneous rate of right atrial preparations with pacemaker shifts from SA node to SAP compared to those (same test condition) without such shifts.

Data shown are mean ± SEM. The number of preparations is given in parentheses.

AUTOMATICITY IN PREPARATIONS WITH PACEMAKER SHIFTS

Pacemaker shifts from the SA node to SAP were observed most commonly under three test conditions: 1) 1% halothane with ouabain 1 \times 10^{-7} M (4 of 14 preparations, table 40); 2) 2% halothane with ouabain 5 \times 10^{-8} M (10 of 34 preparations, table 5); 3) 2% halothane with ouabain 1 \times 10^{-7} M (16 of 25 preparations, table 5). The spontaneous rates of preparations with shifts are compared in table 6 to those without shifts for the same test condition in table 6. These data indicate that the rates of preparations under SAP control are not substantially different from those of preparations under SA node control. Furthermore, the data indicate that in preparations under SAP control, the spontaneous rate is too slow to account for tachycardia mediated by enhanced automaticity of SAP.

PACEMAKER ARREST

Pacemaker arrest with asystole was observed in six preparations during or after exposure to halothane and/or ouabain. The test conditions and a brief description of the events surrounding each of these arrests are provided in table 7. In figure 2, we show sequential tracings depicting events accompanying pacemaker arrest and asystole for one preparation. In most cases, arrest was evidenced by sudden electrical quiescence (at all recording sites). Prior to arrest, the SA node was dominant, with a cranio-caudal activation sequence. In addition, the spontaneous cycle length remained fairly constant prior to arrest; that is, there was no evidence of dropped beats to suggest intermittent SA block. In the remaining preparations, arrest of the SA node was followed by a pacemaker shift to one or more of the SAP sites. Also, in one of these preparations, the spontaneous cycle length was progressively longer with each shift (SA node = 630 ms, SAP site 2 = 720 ms, and SAP site 3 = 950 ms). Once again, however, in the preparations with pacemaker shifts prior to arrest, there were no dropped beats prior to arrest. However, block of conduc tion out of the SA node cannot be ruled out. Tracings from the sixth preparation (fig. 2) suggest that conduction into the SA node from SAP site 1 was blocked, as was conduction to SAP site 3.

Discussion

Our hypotheses were: that increasing ouabain would augment automaticity of SAP compared to the SA node; that such enhanced automaticity might explain digitalis-caused atrial tachyarrhythmias; and that halothane would oppose digitalis-caused atrial tachyarrhythmias by antagonizing ouabain-enhanced automaticity of SAP. These hypotheses were supported and extended by the present study.

Table 7. Pacemaker Arrest and Asystole during or after Exposure to Halothane and/or Ouabain

<table>
<thead>
<tr>
<th>Condition</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% H + 1 \times 10^{-7} O</td>
<td>Sudden SAN arrest; no pacemaker shift; preceding CLs constant</td>
</tr>
<tr>
<td>2% H + 1 \times 10^{-7} O</td>
<td>SAN arrest; shift to SAP-2, then SAP-3, then arrest; CL lengthen prior to arrest</td>
</tr>
<tr>
<td>C_5 control (2% H)</td>
<td>Sudden SAN arrest; no pacemaker shift; preceding CLs constant</td>
</tr>
<tr>
<td>C_5 control (1% H)</td>
<td>Sudden SAN arrest; no pacemaker shift; preceding CLs constant</td>
</tr>
<tr>
<td>C_4 control (0 time control)</td>
<td>SAN arrest; shift to SAP-1 with no conduction to SAN or SAP-3; arrest and asystole; preceding CLs constant</td>
</tr>
</tbody>
</table>

H = halothane; O = ouabain; C_1, C_4, C_5 = respective controls (see fig. 1); SAN = SA node; SAP-1, 2, 3 = SAP electrode sites; CL = spontaneous cycle length.
hypotheses were tested in a perfused canine right atrial preparation, so that any observed effects could be considered direct actions of the drugs tested.

Data we obtained in the perfused canine right atrial preparation negate our hypotheses. Nevertheless, halothane and mid-therapeutic or borderline-toxic levels of ouabain did interact to preferentially increase automaticity in SAP compared to the SA node in many preparations. This is based on the observation of a significant increase in normalized magnitude scores occasioned by shifts in pacemaker location away from the SA node to SAP sites along the sulcus terminals during combined drug exposure. Alternatively, pacemaker shifts to SAP during exposure to ouabain and halothane could occur if, in the presence of ouabain, SA node pacemaker cells were more sensitive to the negative chronotropic action of halothane as compared to SAP cells.

Despite an increase in the automaticity of SAP relative to the SA node in many preparations exposed to ouabain and halothane (tables 4 and 5), as well as an overall increase in the spontaneous rate of preparations with ouabain irrespective of pacemaker location (tables 2 and 3), under no test condition was the rate of preparations sufficient to account for so-called "paroxysmal" atrial tachycardia with block, a common manifestation of digitalis intoxication in humans. A plausible explanation for this, in addition to possible direct depression of SAP automaticity by halothane and lack of autonomic-mediated effects in our preparation, is the selection of an only borderline-toxic concentration of ouabain as the highest dose tested. In preliminary experiments (n = 5), two higher concentrations of ouabain (1 and 2 \times 10^{-5} \text{M}) were tested. Either concentration produced total atrial arrest, preceded by a regular tachycardia (cycle length 200–300 ms) and then irregular beating, within 60–100 min of exposure to ouabain. Similar findings were reported by Steinbeck et al. in an isolated SA node preparation from the rabbit heart. However, we wished to use a concentration of ouabain that would not produce SA-node or SAP arrest (in the absence of halothane) during experiments lasting 4–5 h. Moreover, effects of the highest concentration of ouabain selected for experiments had to be reversible upon washout. As can be seen in time control data for heart rate (table 2), this was achieved for concentrations of ouabain of up to 1 \times 10^{-7}, considered low- or mid-therapeutic, or borderline-toxic by others.

A differential increase in the automaticity of SAP relative to that of the SA node with ouabain and halothane may have several explanations, as follows. 1) Spontaneous diastolic (phase-4) depolarization occurs from a lower (less negative) maximum diastolic potential (MDP) in cells of the SA node compared to SAP and, consequently, may be relatively less sensitive to ouabain-induced Na⁺-K⁺ exchange pump inhibition. 2) Cells of the SA node may be more sensitive than SAP cells to ouabain-induced loss of cell-to-cell electrical coupling and resulting pacemaker desynchronization.

It is possible that SAP have a higher (more negative) maximum diastolic potential than do SA node cells. If so, the ionic basis for automaticity could differ and thereby explain differential effects of ouabain or halothane on automaticity. Nevertheless, the relation between MDP and threshold potential, assuming that the rate of phase 4 depolarization remains constant, may be the most important factor affecting the automaticity of SAP compared to that of the SA node. Thus, we consider it plausible that ouabain-induced inhibition of the Na⁺-K⁺ exchange pump leads to a more pronounced net reduction in MDP in SAP compared to the SA node. The reduction in MDP reduces the time required for

\[ \text{\# The term "paroxysmal" may be a misnomer since basic characteristics of this arrhythmia include gradual onset, rate of 150–220 beats per min, isoelectric baseline between P waves, gradual slowing after withdrawal of digitalis, and various degrees of atrioventricular block. Steinbeck et al. argue convincingly for the term "digitalis-induced sinus" tachycardia with block.} \]

\[ \text{\# The pacemaker current (i) appears to play a more important role in phase-4 depolarization in SAP as compared to SA node cells.} \]

\[ \text{Though less important in SA node cells, calcium release from the sarcoplasmic reticulum may provide a significant component of late phase-4 depolarization in SAP cells.} \]
phase-4 depolarization to bring the SAP cell to threshold potential and thereby enhances automaticity.

In addition, a decrease in MDP by itself can increase the rate of phase-4 depolarization, further augmenting automaticity of SAP relative to the SA node. Thus, automaticity of SAP may have been increased more than that of the SA node, accounting for pacemaker shifts to SAP sites during exposure to ouabain and halothane. Of course, such speculation assumes that halothane reduces the rate of phase-4 depolarization more in the SA node than in SAP. Although the latter assumption is not known (direct comparisons are required in a preparation such as ours), it may be reasonable to speculate so, since halothane has been shown to increase the rate of phase-4 depolarization in spontaneously beating Purkinje fibers (preliminary observations). Both SAP and Purkinje fibers have more negative MDP than do SA node cells and might be expected to have more nearly similar ionic mechanisms for automaticity.

Differential effects of ouabain and halothane automaticity of atrial pacemakers favoring shifts in SEA from the SA node to SAP sites may result from drug-induced SA node pacemaker desynchronization. There is evidence that ouabain produces such an effect in the rabbit SA node, but comparable data for halothane are not available. Results reported by Jalife suggest that rhythm coordination of multiple pacemaker cells in the SA node depends on mutual entrainment as well as on the degree of electrical coupling between cells. Both ouabain and halothane have been shown to reduce electrical coupling between ventricular myocytes. It seems plausible that such uncoupling should extend to atrial myocytes and SA node cells as well, although to our knowledge this has yet to be confirmed.

However, if halothane or ouabain do reduce cell-to-cell coupling at the SA node or between SAP and atrial myocytes, our current findings could be explained as follows. First, the reduction in cell-to-cell coupling is greatest with higher concentrations of ouabain and halothane, explaining the highest incidence of pacemaker shifts and atrial quiescence with higher combined drug concentrations. Second, preferential suppression of SA node compared to SAP automaticity may occur because cells of the SA node are more homogenous, clustered, and mutually interdependent than are SAP and therefore are more vulnerable to uncoupling. The pacemaker process in SAP, in contrast, is possibly not so dependent on mutual entrainment. Rather, as suggested by Rubenstein et al., SAP automaticity is more affected by electrotonic interactions with adjacent, nonautomatic atrial myocytes. If so, and if ouabain and halothane reduce cell-to-cell coupling between atrial myocytes and SAP, then SAP should become less restrained and therefore available to control the atrial rhythm. In addition, it must be assumed (as argued above) that SAP automaticity (spontaneous phase-4 depolarization) is less affected by halothane and ouabain than is SA node automaticity.

Our results may have clinical relevance, but they must be interpreted cautiously, since both halothane and ouabain have significant (indirect) effects on the autonomic nervous system that are not apparent in our model. With this consideration, our findings suggest the following. First, enhanced automaticity of SAP as compared to the SA node does not explain atrial tachyarhythmias with toxic concentrations of digitalis. Likewise, halothane should not be supposed to facilitate or oppose such arrhythmias until more data are available, including relevant in vivo testing. Second, it is possible that high concentrations of digitalis and halothane interact to depress atrial pacemaker activity leading to atrial electrical quiescence. We speculate that this is most likely to occur in patients with sinus node dysfunction due to intrinsic disease, drugs, or impaired autonomic regulation.

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References

12. Rozanski Gj, Jalife J: Automaticity in atrioventricular valve leaflets
43. Toda N, West TC: Modification by sodium and calcium of the cardiac toxicity induced by ouabain. J Pharmacol Exp Ther 154: 239–249, 1966