Significance of the Number of Stimuli to Initiate Ouabain-Induced Arrhythmias in the Intact Heart

Marc A. Vos, Anton P.M. Gorgels, Jet D.M. Leunissen, Ronald T.A.M. van Deursen, and Hein J.J. Wellens

Ouabain-induced arrhythmias are a well-known model used to study triggered activity resulting from delayed afterdepolarizations. In the intact heart, initiation of these arrhythmias is promoted by pacing, especially at fast rates. However, the relevance of the number of stimuli is unknown. In conscious dogs with formalin-induced atrioventricular block, we investigated the effect of variations in pacing mode on 1) the behavior of nonsustained triggered rhythms at progressive levels of ouabain intoxication, and 2) the induction of sustained ventricular tachycardia (VT). Twenty experiments were analyzed. Ouabain was administered as a bolus of 40 μg/kg followed by continuous infusion. Every 15 minutes the pacing protocol was repeated, with a maximum of 10, until completion or induction of VT. When VT could not be initiated, the experiment was repeated at least 1 week later, adding 5–10 μg/kg ouabain to the bolus and increasing the infusion rate correspondingly. This was repeated until VT could be induced. Four interstimulus intervals (200, 400, 600, and 800 msec) and seven numbers of stimuli (5, 10, 20, 35, 50, 100, and 150) were given in two pacing protocols. The effect of these protocols on 1) the number of induced beats per stimulation train, 2) their first postspacing interval, and 3) induction of VT were studied. Initiation of VT occurred after 75±42 minutes. The bolus of ouabain needed to induce VT was inversely related to the body weight of the animals. Progression of ouabain intoxication resulted in 1) a significant increase in the number of induced beats per stimulation train and 2) a significant shortening of the first postspacing interval. Stimulation at a faster rate and/or more stimuli resulted in 1) a significantly pronounced increase in the number of induced beats at the higher levels and 2) a significantly shorter first postspacing interval at successive levels of ouabain intoxication. As a result, VT induction was more frequently observed after pacing with a high number of stimuli (≥50 stimuli, 88%) using short interstimulus intervals (≤400 msec, 100%). In conclusion, VT induction in the presence of ouabain is promoted by pacing using shorter intervals and a higher number of stimuli. (Circulation Research 1991;68:38–44)

During the last two decades, triggered activity resulting from delayed afterdepolarizations (DADs) has received increased attention as a mechanism responsible for cardiac arrhythmias. Usually, programmed electrical stimulation has been used for induction of triggered activity. Irrespective of the cause, ouabain- or catecholamine-induced arrhythmias, pacing rate has been emphasized as an important factor in the initiation of these arrhythmias resulting from DADs. Both in vitro and in vivo studies have shown that shortening of the interstimulus interval results in 1) an increase in DAD amplitude, 2) increased incidence, 3) shortening of the first postspacing interval, and 4) an increased number of induced beats postspacing. The literature, however, is not unanimous on the effect of the number of stimuli on inducing triggered arrhythmias. It has been described that DADs induced by ouabain show an increase in amplitude on increasing the number of stimuli up to 10, but a further increase did not seem to modify the attained DAD amplitude or the length of the coupling interval. On the other hand, catecholamine-induced DADs demonstrated an inverse relation between the number of stimuli and the first postspacing interval and a concordant relation between the number of stimuli and the number of induced triggered beats.
Ouabain-induced tachycardias are a well-known model used to study triggered activity resulting from DADs. To determine the relevance of the number of stimuli for the initiation of these arrhythmias in the intact heart, we investigated the effect of different pacing modes on 1) the behavior of nonsustained arrhythmias at progressive levels of ouabain intoxication and 2) induction of sustained ventricular tachycardia (VT). For this purpose, conscious dogs with surgically induced atrioventricular block were used. This model allows detailed study of ventricular impulse formation without interference by conducted sinus node impulses.

Materials and Methods
Preparation of Study Animals and Methods of Registration

Mongrel dogs with a body weight of 15–37 kg (mean±SD, 25±6 kg) were used. Through a right thoracotomy, complete atrioventricular block was induced by injecting 37% formalin in the region of the bundle of His.11 During this procedure, two electrodes were fixed intramurally into the basal free wall of the right ventricle and the apex of the left ventricle. The electrodes were exteriorized through the skin of the neck. Proper care of the animals was taken during and after surgery according to the requirements of the American Physiological Society.

All animals were studied in the conscious state without premedication. The experiments were performed after a recovery period of 2 weeks to avoid episodes of spontaneous VTs, which are known to occur after creation of atrioventricular block.12,13 Six electrocardiographic leads and one local electrogram were registered simultaneously. Pacing was performed mostly on the right ventricle using a programmable stimulator with a synchronizing circuit. Unipolar stimuli were applied using a stimulus strength of twice diastolic threshold. With a computerized QRS complex detection system,14 values of RR intervals were displayed instantaneously on a monitor screen. Before infusion of ouabain, all stimulation trains used in the pacing protocol were given at least once under control conditions.

Initiation Protocol

Ouabain was infused continuously (0.072 μg/kg/min) after a loading dose of 40 μg/kg i.v. administered in 1 minute.15 Because ouabain reaches a stable plasma concentration after 30 minutes, the experiments in which VT occurred within this period were excluded from analysis.15 Stimulation was started 15 minutes after the loading dose of ouabain.

The complete stimulation protocol was given within 15 minutes and repeated maximally 10 times per experiment. When this protocol failed to induce VT, the experiment was repeated at least 1 week later using a larger loading dose of ouabain (increment of 5–10 μg/kg) and a higher corresponding continuous infusion rate (for each 5 μg/kg of ouabain extra, the rate was increased by 0.009 μg/kg/min) until VT was induced.

During pacing, both the interstimulus intervals and the number of stimuli were varied. After each stimulation train, enough time (45–90 seconds) was allowed for recovery of the rhythm to the prepacing rate. Two pacing protocols were used. In protocol A (designed to study the effect of changing the interstimulus interval), one set of eight stimulation trains with four different interstimulus intervals (200, 400, 600, and 800 msec) was given using 10 or 50 stimuli. In protocol B (designed to study the effect of changing the number of stimuli), 14 stimulation trains were delivered using 5, 10, 20, 35, 50, 100, and 150 stimuli with interstimulus intervals of 200 or 400 msec. The sequence of stimulation trains per set was random.

Data Analysis

To quantify the effect of interstimulus intervals and number of stimuli, these parameters were related to 1) the number of induced beats per stimulation train, 2) their first postpacing interval, and 3) the induction of sustained VT. For the purpose of this study, an induced ectopic beat was defined as a QRS complex having a first postpacing interval shorter than the last spontaneous idioventricular interval prepacing. Ouabain-induced VT was defined as an arrhythmia having a cycle length shorter than 600 msec and consisting of at least 150 beats.

Because the dogs showed a wide variation in the time necessary for VT to be induced (range, 32–146 minutes), the time to onset of VT was divided in five percentual time periods consisting of 20% intervals: 0–20%, 20–40%, 40–60%, 60–80%, and 80–100% of the time interval from the beginning of pacing to onset of VT. Each normalized time period lasted 15±8 minutes.

Data concerning the effect of the four interstimulus intervals on inducing triggered rhythms in protocol A were analyzed by taking the two stimuli together, because this parameter was studied in protocol B. Similarly, to compare the effect of the number of stimuli, the data of protocol B were presented without differentiating between the two interstimulus intervals used. To increase the number of data in each group and to prevent the possibility that data presentation would become too extensive and complex, we combined the data of protocol B into four groups: 5–10, 20–35, 50, and 100–150 stimuli.

Statistical Analysis

Analysis of variance, Student’s t test for unpaired events, χ2 testing, and linear regression analysis were applied to determine statistical significance. Values are expressed as mean±SD.

Results

Twenty-four experiments in which VT occurred were analyzed. We had to exclude data from four animals because of spontaneous onset of VT (i.e., within 15 minutes postpacing or within 30 minutes
after the bolus of ouabain was given). In the 20 remaining experiments, VT was induced after 75±42 minutes of pacing. The mean time for each of the five normalized time periods was 15±8 minutes. Thus, the first period ended after 15±8 minutes and the last period just before VT extended from 60±33 to 75±42 minutes.

Figure 1 presents an example of VT initiation. It is shown that using the same stimulation train, progression of ouabain intoxication (expressed in time to onset of VT and corresponding percentage level) results in 1) an increase in the number of induced beats per stimulation train and 2) a reduction of the first postpacing interval.

A mean bolus of 46±6 µg/kg ouabain was administered. Our minimum dosage of ouabain given (40 µg/kg) was sufficient to induce VT in dogs with body weights more than 30 kg. However, most of the dogs with lower body weights had to be returned to the laboratory for a second or even a third test. Linear regression analysis demonstrated that a significant inverse relation existed (p<0.01) between the two variables (Figure 2). Protocol A consisted of 407 stimulation trains in 11 experiments, and 464 stimulation trains were given in protocol B. Quantification of the findings led to the following results.

Effect of Ouabain Intoxication on the Idioventricular Rhythm

Directly after the bolus of ouabain, a marked increase in spontaneous RR cycle length of the idioventricular rhythm was noticed from 1,310±330 (control) to 1,460±310 msec (p<0.001). Thereafter, a gradual decrease in spontaneous cycle length occurred to 1,250±240 msec just before onset of VT (period 80–100%), this value being not significantly different from the control value.
**Effect of Progressive Ouabain Intoxication on Number of Induced Beats and the First Postpacing Interval**

Tables 1–4 present the results of the two protocols on the number of induced beats and length of the first postpacing interval. Independent of the variations in pacing, ouabain intoxication (columns) resulted in 1) a significant increase in number of induced beats per stimulation train (Tables 1 and 3) and 2) a significant reduction in the length of the first postpacing interval (Tables 2 and 4). The only exception was pacing with an interstimulus interval of 800 msec (Tables 1 and 2).

**Effect of Shortening Interstimulus Interval in Relation to Different Levels of Ouabain Intoxication**

Only at the highest level of ouabain intoxication (Table 1, row 80–100%) was the number of induced beats per stimulation found to be related to the interstimulus interval. At that level a reduction of the interstimulus interval from 800 to 200 msec resulted in a significant increase in the number of induced beats (p≤0.001).

The first postpacing interval (Table 2) significantly reduced at all levels of intoxication (p≤0.001) on shortening the pacing intervals from 800 to 200 msec. Induction of VT also was dependent on interstimulus interval (p≤0.001). It was never observed after 600 and 800 msec but was observed after interstimulus intervals of 400 (in six of 11 experiments) and 200 msec (in the remaining five experiments).

**Effect of Increasing the Number of Stimuli in Relation to Different Levels of Ouabain Intoxication**

Only at the higher levels (≥60%) of ouabain intoxication did we observe a significantly more pronounced increase in number of induced beats per stimulation train on increasing the number of stimuli from 5–10 to 100–150 (Table 3). Independent of the level of intoxication (Table 4), it was found that the first postpacing interval of the triggered beats significantly decreased (p≤0.001) on increasing the number of stimuli from 5–10 to 100–150.

Initiation of VT was observed only once when pacing was performed with fewer than 50 stimuli, that is, after 5–10 stimuli. No difference was seen between the higher number of stimuli (after 50 stimuli in four of nine experiments, and after 100–150 stimuli in the remaining four experiments). Therefore, induction of VT was dependent on the number of stimuli (p≤0.001).

**Discussion**

It is known that several hours can elapse before ouabain induces ventricular arrhythmias in the intact heart.10 Pacing at faster rates can accelerate the occurrence of arrhythmias5,17–19 or will enable induc-

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**Table 1. Effect of Shortening Interstimulus Interval on Number of Ectopic Beats per Stimulation Train During Progressive Ouabain Intoxication**

<table>
<thead>
<tr>
<th>Time to VT (%)</th>
<th>Interstimulus interval (msec)</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td>0–20</td>
<td>1.8±1.7*</td>
<td>2.3±2.1*</td>
</tr>
<tr>
<td>20–40</td>
<td>3.7±7.6*</td>
<td>3.2±4.2*</td>
</tr>
<tr>
<td>40–60</td>
<td>4.3±8.6*</td>
<td>3.0±2.5*</td>
</tr>
<tr>
<td>60–80</td>
<td>9.0±9.3*</td>
<td>10.0±11.9*</td>
</tr>
<tr>
<td>80–100</td>
<td>54.4±42.7</td>
<td>54.0±39.2</td>
</tr>
</tbody>
</table>

Values are mean±SD. Time to VT, time interval in percentages to induce sustained ventricular tachycardia; NS, not significant.

*p<0.05 compared with 80–100%.

*tp<0.01 compared with 200 msec.

**Table 2. Effect of Shortening Interstimulus Interval on First Postpacing Interval During Progressive Ouabain Intoxication**

<table>
<thead>
<tr>
<th>Time to VT (%)</th>
<th>Interstimulus interval (msec)</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td>0–20</td>
<td>790±250*</td>
<td>870±300*</td>
</tr>
<tr>
<td>20–40</td>
<td>620±170*</td>
<td>720±200*</td>
</tr>
<tr>
<td>40–60</td>
<td>630±190*</td>
<td>670±200*</td>
</tr>
<tr>
<td>60–80</td>
<td>520±160*</td>
<td>560±100*</td>
</tr>
<tr>
<td>80–100</td>
<td>470±110*</td>
<td>480±90</td>
</tr>
</tbody>
</table>

Values are mean±SD. Time to VT, time interval in percentages to induce sustained ventricular tachycardia; NS, not significant.

*p<0.05 compared with 80–100%.

*tp<0.01 compared with 200 msec.
tion of tachycardias at lower dosages of glycosides. In this study, the relevance of the number of stimuli was investigated. It was found that similar to pacing with fast rates, an increase in the number of stimuli resulted in a more pronounced increase in induced beats (at the higher levels of ouabain intoxication), a shorter first postpacing interval (at successive levels of ouabain intoxication), and 3) induction of VT. We used an animal model consisting of conscious dogs with chronic, complete atrioventricular block. This allows study of arrhythmias at the ventricular level, without interference of normally conducted sinus beats. Using the combination of ouabain and programmed electrical stimulation, we found that the dosage of ouabain required to induce VT was inversely related to body weight. Such a relation is common in species having marked differences in body size. Because of a higher level of metabolism, a smaller animal needs a higher dosage of medication to achieve a similar effect. In the same kind of species, however, such a relation is to our knowledge never described. Taking the heart rate as an indicator of the level of metabolism in our dogs, we did not observe any difference between dogs with the lowest (15 kg) or with the highest (37 kg) body weight. Therefore, we exclude the level of metabolism as an explanation for the observed difference. When speculate, we have thought of the volume of blood circulating or the number of receptor cells of the Na⁺,K⁺-ATPase as other possible factors.

Several authors have quantified the significance of the pacing rate to induce DAD-dependent arrhythmias in vitro. In the intact heart, it also was demonstrated that a concordant relation exists between the first postpacing interval and the interstimulus intervals. Similarly, in this study we noticed that this concordant relation was present, and we found it to be independent of the level of ouabain intoxication (Table 2). The expected concomitant increase in number of induced beats on reducing the interstimulus interval was, however, only seen at high levels of ouabain in the period just preceding the induction of VT (80–100%, Table 1).

Another interesting finding was the effect of a high number of stimuli on inducing VT. In contrast to the rate of pacing, this variable has not been studied in the intact heart and rarely in vitro. In ouabain-induced DADs, an increase in DAD amplitude has been described for up to 10 stimuli. A further increase in the number of stimuli did not result in a higher incidence of the triggered beats or in a shorter first postpacing interval of the subthreshold DAD. This is in contrast to a study by Wald and Waxman, who found that in catecholamine-induced DAD, increasing the number of stimuli resulted in a shorter first postpacing interval and an increase in the number of induced beats. In our study, we made similar observations. Increasing the number of stimuli from 5–10 to 100–150 resulted in a more pronounced increase in the number of induced beats,

### Table 3. Effect of Increasing Number of Stimuli on Number of Ectopic Beats per Stimulation Train During Progressive Ouabain Intoxication

<table>
<thead>
<tr>
<th>Time to VT (%)</th>
<th>No. of stimuli</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5–10</td>
<td>20–35</td>
</tr>
<tr>
<td>0–20</td>
<td>1.6±1.0*</td>
<td>1.4±0.5*</td>
</tr>
<tr>
<td>20–40</td>
<td>1.9±1.5*</td>
<td>1.9±1.3*</td>
</tr>
<tr>
<td>40–60</td>
<td>1.8±0.9*</td>
<td>3.1±3.5*</td>
</tr>
<tr>
<td>60–80</td>
<td>2.5±1.6*</td>
<td>6.3±12.3*</td>
</tr>
<tr>
<td>80–100</td>
<td>12.8±18.9</td>
<td>22.3±22.0</td>
</tr>
</tbody>
</table>

Analysis of variance:
- p<0.01 compared with 80–100%.
- tp<0.01 compared with 5–10 stimuli.

Values are mean±SD. Time to VT, time interval in percentages to induce sustained ventricular tachycardia; NS, not significant.

### Table 4. Effect of Increasing Number of Stimuli on First Postpacing Interval During Progressive Ouabain Intoxication

<table>
<thead>
<tr>
<th>Time to VT (%)</th>
<th>No. of stimuli</th>
<th>Analysis of variance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5–10</td>
<td>20–35</td>
</tr>
<tr>
<td>0–20</td>
<td>960±210*</td>
<td>760±180*†</td>
</tr>
<tr>
<td>20–40</td>
<td>1,020±230*</td>
<td>700±190*†</td>
</tr>
<tr>
<td>40–60</td>
<td>920±200*</td>
<td>600±160*†</td>
</tr>
<tr>
<td>60–80</td>
<td>900±260*</td>
<td>620±180*†</td>
</tr>
<tr>
<td>80–100</td>
<td>730±270</td>
<td>530±120†</td>
</tr>
</tbody>
</table>

Analysis of variance:
- p<0.01 compared with 80–100%.
- tp<0.01 compared with 5–10 stimuli.
2) a shorter first postspacing interval, and 3) an increased likelihood of VT being induced.

Assuming that ouabain-induced arrhythmias are based on DADs, we offer some explanation for the described discrepancies. Differences between activation or inactivation of the Na⁺,K⁺-ATPase under in vitro and in vivo circumstances could be one of them. Normally, pacing will result in overdrive suppression.23,24 A toxic (high) dosage of ouabain will lead to inactivation of the Na⁺,K⁺-ATPase, increased intracellular sodium concentration, intracellular calcium overload by the Na⁺-Ca²⁺ exchange, and the induction of DADs and possibly triggered beats.25 It very well could be that the degree of inhibition of the Na⁺,K⁺-ATPase in vitro markedly exceeds that ever attained in the intact heart. In support of this hypothesis is the fact that such observations as severe diastolic depolarization, impairment of conduction, and loss of excitability often seen in vitro as a result of ouabain intoxication are not produced in vivo.1 It seems conceivable, therefore, that the number of paced beats will be a decisive factor only when the Na⁺,K⁺-ATPase is only partially inactivated by ouabain.

The way the stimulation protocol is performed could be another variable influencing the level of activity of the Na⁺,K⁺-ATPase. In particular, the rest period between stimulations can be important. In the isolated tissue, the trains of stimuli were separated by only 3-second pauses, whereas we interrupted stimulation for 45–90 seconds. When the latter protocol is performed in vitro, the DAD amplitude is greatly depressed and only recovers after longer periods of pacing (G.R. Ferrier, personal communication, 1990). Thus, the relevance of the number of paced beats affecting the DAD amplitude and the likelihood of the DAD reaching threshold changes according to the quiescence between stimulation trains.

In the intact heart, additional factors may contribute to the initiation of these arrhythmias. Catecholamines or, less specifically, the activity of the autonomic nervous system have to be considered.26,27 This is supported by the observation that in anesthetized dogs, the amount of ouabain (40–50 µg/kg) capable of initiating VT in our experiments results in only single ectopic beats.19,26 It is in this setting that factors creating the greater burden for the Na⁺,K⁺-ATPase, such as a higher number of stimuli, may be capable of inducing (earlier) triggered beats and arrhythmias. The overdrive suppression still seen in our experiments after the induction of triggered beats (Figure 1) is in agreement with the hypothesis that the Na⁺,K⁺-ATPase is still (partially) active.

When the above described model of triggered activity is more representative for the clinical situation, it opens new possibilities for the application of electrical stimulation to identify mechanisms of arrhythmias, especially those leading to triggered activity resulting from DADs. Our results indicate that the induction of a tachycardia after a (prolonged) acceleration in rate suggests a DAD-dependent arrhythmia, whereas the initiation by extrastimuli is in favor of reentry as the arrhythmogenic mechanism.28 In conclusion, induction of VT in the presence of ouabain is promoted by pacing using shorter intervals and a higher number of stimuli.

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