Pacing-Induced Automaticity in Sheep Purkinje Fibers

ROBERT W. WALD AND MENASHE B. WAXMAN

SUMMARY The induction and decay of pacing-induced automaticity were studied in 15 sheep Purkinje fiber preparations superfused with modified Tyrode's solution containing norepinephrine, $2.5 \times 10^{-7}$ M. All preparations were quiescent prior to pacing. Spontaneous automaticity could be induced in each preparation provided a sufficient number of pacing stimuli were applied at a fast enough rate. Once the minimum pacing requirements for induction of automaticity were exceeded, the number, total duration, and fastest rate of the induced beats were proportional to the number and rate of the pacing stimuli up to a maximum which could not be exceeded. Consecutive trains of stimuli were additive in inducing automaticity, provided that the pauses between them were short enough to preclude the time-dependent return of automatic properties to their pre-pacing level. Prolonged sequences of fixed pacing trains resulted in stable degrees of automaticity which depended on the length of the pause between them. These observations permit a semiquantitative description of induced automatic behavior and help establish a model that may be useful in future studies. Circ Res 48: 531-538, 1981

PACING-INDUCED automaticity has been demonstrated in the specialized ventricular conduction tissues of several animal species under a variety of pharmacological and altered ionic conditions (Vassalle and Carpentier, 1972; Davis, 1973; Hogan et al., 1973; Ferrier and Moe, 1973; Cranefield and Aronson, 1974). This phenomenon, which is counter to the previously described suppression of Purkinje fiber automaticity by pacing (Alanis and Benitez, 1967; Vassalle, 1970), is thought to arise by a mechanism which is fundamentally different from that which underlies classical spontaneous automaticity in Purkinje fibers (Ferrier et al., 1973; Rosen et al., 1973a; Ferrier, 1977). When induced in the presence of cardiotonic steroids, the degree of this automaticity has been shown to increase with the rate and duration of stimulation (Davis, 1973; Hogan et al., 1973; Ferrier et al., 1973; Ferrier, 1977). This suggested that this phenomenon was responsible for some pacing-sensitive ventricular arrhythmias which develop in vivo during digitalis intoxication (Vassalle et al., 1962; Wittenberg et al., 1970; Wittenberg et al., 1972). The temporal correlation of ouabain-induced delayed afterdepolarizations in vitro with the appearance of ventricular arrhythmias in vivo was strongly supportive of this relationship (Rosen et al., 1973b).

First described by Vassalle and Carpentier (1972), pacing-induced automaticity, or, as the authors termed it, “overdrive excitation” in lamb Purkinje fibers in a normal ionic environment and in the presence of norepinephrine, $8.8 \times 10^{-7}$ M, represents one of the conditions closest to physiological under which the phenomenon has been described in ventricular tissues. Vassalle et al. (1976b) have demonstrated that overdrive excitation related ventricular arrhythmias in the heart-blocked dog in vivo was dependent on shortening of the diastolic interval and was faster when the number of paced beats was increased. However, detailed studies of its induction and decay in vivo have not hitherto been reported. In the present paper we report the results of studies aimed at characterizing the induction and decay of pacing-induced automaticity in sheep Purkinje fibers superfused with physiological solution in the presence of norepinephrine in concentrations insufficient to cause spontaneous automaticity. These studies were undertaken in an attempt to first develop a semiquantitative description of the phenomenon; second, provide a basis for comparison to observations obtained in other models of stimulation-induced automaticity such as cardiotonic steroid intoxication; and finally, to enlarge the data base from which some in vivo arrhythmias could in future be inferred to arise by this mechanism in a manner similar to the inferences involving the ventricular arrhythmias of digitalis toxicity.

Methods

Purkinje fiber preparations consisting of the proximal segment of the anterior division of the left bundle branch from freshly excised sheep hearts obtained from an abattoir were mounted in a tissue bath (capacity 15 ml) and superfused (30 ± 2 ml/min) with oxygenated (95% O$_2$, 5% CO$_2$) modified Tyrode's solution (NaCl, 137; KCl, 4.0; MgCl$_2$, 0.5; NaH$_2$PO$_4$, 1.8; NaHCO$_3$, 12; CaCl$_2$, 1.35; dextrose, 10).
5.5 mM) at 37 ± 0.5°C. Norepinephrine (Levophed, Winthrop) dissolved in oxygen-free distilled water, brought to pH 4 with HCI to prevent alkaline oxidation, was infused into the stream of superfusate with a constant infusion pump at a rate calculated to yield a final concentration of 2.5 × 10^-7 M. The preparations were paced via bipolar silver surface electrodes using rectangular pulses 1.5x threshold voltage and 2 msec in duration. Pulses isolated from ground were generated by a Grass SD9 stimulator. Accurate sequences of stimulating trains were generated using two Digitimer model 4030 quartz crystal-controlled timing devices. Intracellular action potentials were recorded using standard glass microelectrodes filled with 3.0 M KCl (tip resistance 10-30 MΩ) connected via a Ag-AgCl junction to a Grass P18 DC microelectrode preamplifier with high input impedance and capacity compensation. Action potentials were displayed on a Tektronix 565 oscilloscope, along with their first order derivative. Action potential amplitude and the maximum rate of rise of phase 0 (V max) were measured either directly from the oscilloscope screen or from Polaroid photographs. The rate of spontaneous activity was measured using a Hewlett-Packard model 5326B digital ratemeter. Hard copy recordings of all experiments were obtained using a Siemens Minigograph 800 ink-jet recorder.

Fifteen out of a total of 65 preparations fulfilled the following criteria: (1) they showed no spontaneous automaticity without pacing prior to or following addition of norepinephrine, 2.5 × 10^-7 M, to the superfusate; (2) automaticity could not be induced by pacing for 10 minutes at a cycle length of 300 msec prior to the addition of norepinephrine; and (3) after addition of norepinephrine, they became automatic only after being paced and returned to a quiescent state within less than 15 minutes after cessation of pacing in each instance. The observations obtained from these 15 preparations form the basis of this report.

After addition of norepinephrine, 2.5 × 10^-7 M, to the superfusate and after fulfilling the above criteria, the total number of induced beats, the duration of induced automaticity, and the shortest interval between induced beats were determined as a function of the number and rate of applied pacing stimuli. This was achieved by varying the number of pacing stimuli applied at a cycle length (CL) of 300 msec between 50 and 1000 in five preparations and by varying the cycle length of 1000 applied stimuli between 300 and 1000 msec. An interval of at least 3 minutes was allowed to elapse following cessation of induced automaticity, and at least two observations were recorded with each pacing train.

Five other preparations were subjected to a portion of the above protocol. Following this, the effect of 10- to 120-second pauses between fixed sequences of trains consisting of up to 100 stimuli applied at a cycle length of 300 msec was studied to assess the rate of time-dependent decay in automatic properties.

Results

Figure 1 illustrates the induction of automaticity in a quiescent preparation by 150 pacing impulses applied at a basic cycle length (BCL) of 400 msec. Following cessation of pacing, the induced automaticity accelerated initially, then slowed gradually and returned to quiescence after 2.5 minutes of activity. Resting membrane potential prior to pacing was -76 mV. Maximum diastolic potential during pacing increased from -85 to -90 mV while action potential amplitude was 110 mV.

![Figure 1](image1.png)

**Figure 1** Induction of automaticity in an initially quiescent sheep Purkinje fiber preparation by 150 pacing impulses applied at a basic cycle length (BCL) of 400 msec. Following cessation of pacing, the induced automaticity accelerated initially, then slowed gradually and returned to quiescence after 2.5 minutes of activity. Resting membrane potential prior to pacing was -76 mV. Maximum diastolic potential during pacing increased from -85 to -90 mV while action potential amplitude was 110 mV.

![Figure 2](image2.png)

**Figure 2** Effect of increasing numbers of applied pacing impulses. A fiber initially quiescent (control) at a concentration of norepinephrine of 2.5 × 10^-7 M failed to exhibit induced automaticity following 50 stimuli applied at a cycle length (CL) of 250 msec but became increasingly automatic following 100 and 200 stimuli, respectively. (Only the last few pacing cycles are displayed in each tracing.) Small oscillations in membrane potential can be seen to precede the induced action potentials in this record except where they are masked by preceding action potentials.
study exhibited this type of behavior in response to pacing.

**Resting Membrane and Action Potential Characteristics**

Under conditions of superfusion with norepinephrine, 2.5 × 10⁻⁷ M, the resting membrane potential of the 15 preparations during quiescence was 77.3 ± 7.2 mV (mean ± sd). During stimulation at CL 2000 msec, action potential amplitude was 113.8 ± 10.2 mV and V_max was 344.7 ± 69.4 V/sec.

Small subthreshold oscillations in resting membrane potential were observed frequently during most but not all experiments (Figs. 2, 5, and 6). In a few instances, these oscillations clearly preceded the induced action potentials (Fig. 2).

### Table 1: Effect of Number of Stimuli Applied at Cycle Length 300 msec on Pacing-Induced Automaticity

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</table>

Results are expressed as mean ± sem. Numbers in parentheses represent the respective number of observations. PIB’s = pacing-induced beats; Duration = total duration of induced automaticity; Shortest CL = shortest cycle length of induced activity; NIL = no induced beats.

* Automaticity induced by one of two trials.

** Computed only for complete columns, SEM unweighted.
to quantify the degree of induced automaticity. Using these criteria, the degree of induced automaticity increased with increasing numbers of applied stimuli (Table 1; Figs. 2, 3). This relationship was statistically significant even when a straight linear fit was applied to the data from each preparation \( P < 0.05 \). If a more complex relationship is present, such an analysis places the data at a disadvantage. The data pooled from all five preparations analyzed by Friedman's two way non-parametric analysis of variance also established this relationship to be highly significant \( P < 0.005 \).

The relationship between the above-noted parameters of induced automaticity and the rate of applied impulses was determined in a separate set of experiments performed in five preparations. In these experiments, 1000 stimuli were applied to the preparation at CL's ranging from 300 to 1000 msec. The results are summarized in Table 2 and the data from a representative experiment are illustrated in Figure 4. The number of induced beats and the duration of induced automaticity varied inversely with the stimulus CL, whereas the shortest induced CL varied directly with the stimulus CL. These relationships were again highly significant when analyzed by the abovementioned non-parametric technique \( P < 0.005 \).

**Additive Effects of Successive Trains of Stimulation**

As mentioned previously, a minimum number of pacing impulses at any given rate of stimulation always was required to induce automaticity in a preparation. This minimum number of impulses was constant for any given preparation provided that a long period of time was allowed to elapse after the preceding stimulus challenge. When the number of pacing impulses applied was less than that required to induce automaticity, a summation of properties leading to automaticity was demonstrated by the appearance of spontaneous beats following the sequential application of several trains of pulses, none of which individually would induce automaticity in a previously quiescent preparation. Figure 5 illustrates this observation in a preparation that had been quiescent for 10 minutes. Application of 50 stimuli at a CL of 300 msec resulted in no automaticity. A second, then a third identical train of stimuli applied 15 seconds after the previous train resulted in increasing numbers of induced beats. This behavior was observed in all 15 preparations used.

**Time-Dependent Decay of Induced Automaticity**

To demonstrate the time-dependent decay of automatic properties, several experiments were performed using constant pacing trains applied at regular intervals with the only variable being the pause between successive trains. The pauses between trains were varied only after the automatic behavior of the fiber had stabilized over at least 10 consecutive pacing trains. In this manner it was determined that the same pacing trains could result in a build-up, maintenance, or decay of induced automaticity, depending on the length of the pause between consecutive trains. Figure 6 illustrates discontinuous portions of such an experiment. In this instance, trains of 20 stimuli at a CL of 300 msec separated by pauses of 15 seconds were insufficient to induce automaticity (first strip) but were able to maintain a steady degree of automaticity (third strip) that had been induced by decreasing the pauses between trains to 10 seconds (second strip). Finally, when the pauses between trains were increased to 20 seconds (fourth strip), automaticity gradually subsided. The effect of trains of 20, 50, and 100 stimuli separated by 10, 30, 60, and 120 second pauses was studied systematically in five preparations. The results are illustrated in Figure 7. When the pause between trains was 120 seconds, none of the preparations exhibited a build-up of automaticity, whereas 10-second pauses resulted in additive effects in all preparations when stimulated with trains of 50 or 100 stimuli.
Table 2  Effect of Cycle Length of 1000 Stimuli Pacing-Induced Automaticity

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<tr>
<th>Preparation no.</th>
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| Results are expressed as mean ± SEM. Numbers in parentheses represent the respective number of observations. PIB's = pacing-induced beats; Duration = total duration of induced automaticity; Shortest CL = shortest cycle length of induced activity. |

Discussion

We can summarize our findings regarding the induction and time-dependent decay of pacing-induced automaticity in sheep Purkinje fibers superfused with norepinephrine as follows. (1) There was a direct relationship between the degree of induced automaticity as assessed by the number of induced beats, their duration and fastest rate, and the number and frequency of applied stimuli up to a maximum which could not be exceeded. (2) Induced automaticity appeared to result from a yet undefined change in membrane properties acquired dur-

Figure 4  Effect of CL of 1000 pacing stimuli on the number of induced beats (top), the duration of induced automaticity (middle) and the shortest induced CL (bottom). All points represent the mean and standard error of two or more separate determinations.

Figure 5  Additive effects of successive stimulating trains. This preparation has been quiescent for 10 minutes prior to being stimulated. Trains of 50 stimuli (CI 300 msec), insufficient to induce automaticity when applied individually, elicit automaticity when applied in succession at regular intervals of 30 seconds. Note that oscillatory activity is present in the record of membrane potential after each train of stimuli but fails to reach threshold after the first train.
Effect of varying the pauses between constant trains of 20 stimuli applied at a BCL of 300 msec on induced automaticity. The four strips are not continuous but are in chronological order. See text for details of the experimental protocol. Pauses of 15 seconds between consecutive trains did not allow induction of automaticity during a 20-minute period of continuous testing (top strip). Less than 2 minutes after reduction of the pause length to 10 seconds, automaticity emerged and became rapidly enhanced (second strip). When the pauses were again increased to 15 seconds, the number of induced beats decreased and became stabilized within 4 minutes (third strip). When the pauses were further increased to 20 seconds, induced automaticity rapidly disappeared (fourth strip).

Effect of altering the duration of the pause between trains of 20, 50, and 100 stimuli (CL 300 msec) on the behavior of induced automaticity in five preparations. Bars above and below the horizontal line represent the number of preparations in which induced automaticity was respectively enhanced or decayed during repeated application of the respective trains of stimuli separated by the indicated pauses. Preparations which showed no change at any given pause were excluded from this illustration.

The conditions under which the studies reported here were carried out resemble those used by Vassalle and Carpentier (1972) who demonstrated pacing-induced automaticity in lamb Purkinje fibers superfused with Tyrode's solution of normal physiological ionic composition in the presence of noradrenaline, $8.8 \times 10^{-7}$ M. Although only 15 of a total of 65 preparations met the criteria for inclusion into this study, pacing-induced enhancement of automatic properties was observed in most of the remaining preparations as well. Most of these were discarded because of either random or regular background automaticity which was unrelated to pacing and which made it impossible to quantify the effects of pacing in the manner required by our protocol.

None of the preparations used in our study exhibited pacing-induced automaticity in the absence of noradrenaline. Therefore noradrenaline has an obligatory role in this phenomenon. The role of adrenergic enhancement in overdrive excitation also has been studied in vivo in dogs by Vassalle et al. (1976a). They demonstrated fast ventricular rhythms induced by rapid pacing in dogs with complete atrioventricular block during stimulation of extrinsic stimulation. This change could be shown to be occurring in response to stimulation even when overt automaticity had not yet emerged and was cumulatively additive over successive trains of stimuli, provided the pause between trains was short enough. This change in membrane properties had to reach a minimal level which one might term "automaticity threshold" before automaticity became overtly manifest. (3) Following cessation of stimulation, there was a time-dependent decay in automaticity as well as in the above-mentioned underlying change in membrane properties.

The fact that the degree of induced automaticity could not be increased over a certain maximum by increasing the number of applied beats suggests that, as the level of automaticity increases, either the capacity of additional stimuli to increment it decreases further, or the rate of its time-dependent decay increases, or a combination of these prevails. Thus it appears that, once this maximal degree of automaticity is reached for a given frequency of stimulation, the amount of turn-on caused by each stimulus is equal to the decay between consecutive stimuli. The theoretical saturation point of this process cannot be tested because the fastest stimulating frequency is limited by the refractory period of tissue. All of these considerations are also applicable to the induction and decay of automatic properties during the application of repetitive trains separated by constant pauses. The advantage of the latter is that the degree of automaticity may be sampled during each pause without interrupting the pattern of stimulation. A steady state condition such as in the third strip in Figure 6 is achieved when the extent of automaticity induced by each train of stimuli is equal to the decay of automatic properties during the ensuing pause.

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the left stellate ganglion or administration of nor-
epinephrine. The rate of induced rhythm was ac-
celerated by further drive and the arrhythmogenic
effect was enhanced by increasing the [Ca\textsuperscript{2+}].

Working with the same experimental model, Vas-
salle et al. (1976b) also have shown that overdrive
excitation-related ventricular arrhythmias were
more likely to be induced by faster driving rates
and were more rapid with increasing numbers of
driving stimuli. Our in vitro experimental results
are in complete agreement with these in vivo ob-
servations.

Vassalle and Carpentier (1972) have postulated
that overdrive excitation may be attributed to a
simultaneous combination of: (1) a norepinephrine-
mediated shift in the steady state activation curve
of the slow K\textsuperscript{+} current, i_{K2} (Noble and Tsien, 1968),
in a depolarizing direction (Hauswirth et al., 1968)
and (2) an overdrive-mediated (Vassalle, 1970) nor-
epinephrine-enhanced (Carpentier and Vassalle,
1971; Vassalle and Carpentier, 1971) increase in
maximum diastolic potential. They felt that hyper-
polarization accelerated the pacemaker potential in
two ways—first, by lowering the steady state value
of s\textsubscript{a} and thereby increasing the s-s\textsubscript{a} difference at
the beginning of diastole, and second, by increasing
the rate constant of inactivation of i_{K2}, which is
higher at membrane potentials negative to about
—75mV. The relative importance of these two fac-
tors varies in opposite directions at different nor-
epinephrine (NE) concentrations. The shift in the
steady state activation curve of i_{K2} in a depolarizing
direction under the influence of NE means that s\textsubscript{a}
is fully inactivated at —80 mV [NE = 10\textsuperscript{-6} M (Tsien,
1974)], and hyperpolarization beyond this potential
results in little or no further increment in the s-s\textsubscript{a}
difference. At the same time, however, Tsien (1974)
has shown that, whereas NE 10\textsuperscript{-8} M has no apparent
effect, 10\textsuperscript{-6} M increases the rate constant of i_{K2},
inactivation 2- to 3-fold at any given potential neg-
ative to about —70 mV. Thus the effect of hyper-
polarization on the rate of inactivation of i_{K2} is
enhanced by NE. These observations probably
would apply even if a decreasing i_{K2} was not solely
responsible for the pacemaker potential in Purkinje
fibers, since they would be equally applicable to
the behavior of an increasing inward current. Our
results would support the hypothesis of Vassalle and
Carpentier (1972) regarding overdrive excitation
provided that it could be shown that the process of
hyperpolarization exhibits a relationship similar to
pacing rate, duration, and pause length, as did in-
duced automaticity in our studies. In studies on
feline Purkinje fibers, Browning et al. (1979) have
demonstrated a linear relationship between steady
state hyperpolarization of maximal diastolic poten-
tial and frequency of stimulation at CL 250 to 2000
msec. Furthermore, hyperpolarization increased
with increasing duration of stimulation at CL 250
msec and decayed exponentially following cessation
of stimulation. Our observations are compatible
with similar relationships for induced automaticity
and are therefore also in agreement with Vassalle
and Carpentier's (1972) hypothesis.

Vassalle et al. (1976a) also have postulated that
overdrive excitation may be related to Ca\textsuperscript{2+} influx
into cells. The relationship of the phenomenon to
stimulation rate and duration, the obligatory role of
norepinephrine, and its enhancement with in-
creased [Ca\textsuperscript{2+}]\textsubscript{i} (Vassalle et al. 1976a) may support
this hypothesis. Temte and Davis (1967) demon-
strated that increased [Ca\textsuperscript{2+}]\textsubscript{i} enhanced diastolic
depolarization in dog Purkinje fibers in a stimulus
rate-dependent manner. Their published records
show waveforms similar to those later designated
as delayed afterdepolarizations. Although Di-
Francesco and McNaughton (1979) found no evi-
dence that calcium had a direct effect on K\textsuperscript{+} chan-
nels in sheep Purkinje fibers, Izenberg (1977) has
shown that [Ca\textsuperscript{2+}] may affect the amplitude of the
steady state activation curve of i_{K2} in these fibers.

We frequently observed oscillatory potentials fol-
lowing stimulation but are unable to comment on
their role in the genesis of induced automaticity.
Vassalle and Carpentier (1972) also have observed
such oscillations following trains of stimuli that
were insufficient to induce automaticity. The role
of oscillatory afterpotentials in the genesis of pac-
ing-induced automaticity in cardiac glycoside intox-
ication has been well established (Ferrier et al.,
1973; Rosen et al., 1973a; Ferrier, 1977). These
afterpotentials were held responsible for previous
observations of enhanced diastolic depolarization
produced by these agents (Vassalle et al., 1962;
Davis, 1973; Kassebaum, 1963) and also were ob-
served to occur along with triggered automaticity
in Na\textsuperscript{+}-free solution (Cranefield and Aronson,
1974). Although oscillatory potentials have been observed
primarily at low membrane potentials (Davis, 1973;
Hogan et al., 1973; Ferrier and Moe, 1973; Crane-
field and Aronson, 1974; Wit and Cranefield, 1976),
they also have been described at relatively normal
membrane potentials (Cranefield, 1975; Wit and
Cranefield, 1977), such as those prevailing in our
experiments.

Ouabain-induced in vivo ventricular arrhythmias
have been shown to be sensitive to the rate and
duration of pacing (Vassalle et al., 1962; Wittenberg
et al., 1970; Wittenberg et al., 1972). Later, a similar
relationship between the rate and duration of pac-
ing and the development of delayed afterdepolariza-
tions in vitro (David, 1973; Hogan et al., 1973;
Ferrier et al., 1973; Rosen et al., 1973) suggested
that the latter represented a mechanism by which
ouabain-induced arrhythmias could arise. Ferrier
and Moe (1973) showed that the amplitude of tran-
sient depolarizations generated by acetylstrophan-
thidin was proportional to [Ca\textsuperscript{2+}]\textsubscript{i}. The link be-
tween acetylstrophanthidin-induced membrane osc-
ilations and contractility established by Ferrier
(1971) suggests that increased [Ca\textsuperscript{2+}] may be re-
sponsible for oscillatory potentials induced by car-
cardiac glycosides. In the light of this possibility, the similarities in the behavior of pacing-induced automaticity in the presence of cardiac glycosides (Vassalle et al., 1962; Wittenberg et al., 1972; Davis, 1973; Hogan et al., 1973; Ferrier et al., 1973; Rosen et al., 1973) to the behavior of the phenomenon studied in this paper assume added significance.

The observations reported here may allow identification of in vivo ventricular arrhythmias which arise by a similar mechanism. Further quantitative work is needed to define the kinetics and establish a mathematical model of the induction and decay of this phenomenon.

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References


DiFrancesco D, McNaughton PA (1979) The effects of calcium on outward membrane currents in the cardiac Purkinje fiber. J Physiol (Lond) 289: 347–373


Johansen DF (1971) Effects of cardiac glycosides on the potassium permeability via the conductance components gK and gK2. Pfluegers Arch 371: 77–85


Tsien RW (1974) Effects of epinephrine on the pacemaker potential current of cardiac Purkinje fibers. J Gen Physiol 64: 293–319


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