Factors Controlling Pacemaker Action in Cells of the Sinoatrial Node

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Studies of the effects of imposed drive on isolated mammalian sinoatrial (SA) node pacemaker cells were undertaken as a result of the observed effects of rapid drive imposed on in situ pacemakers. Such studies are not new since Gaskell in 1884 observed that tetanic stimulation of the turtle sinus venosus or atria had an inhibitory effect on pacemaker function. In the most recent studies of the problem of pacemaker suppression by imposed drive, intracellular recordings from isolated sinoatrial node tissues were used. For example, West reported that repetitive electrical stimulation of the SA node is followed by a brief asystole accompanied by membrane hyperpolarization of pacemaker fibers and then by a period of accelerated firing. The initial negative chronotropic effect is thought to be due to stimulus-released acetylcholine and the late acceleration to a release of norepinephrine. This explanation of the consequences of imposed drive is supported by the observations of Hutter and Trautwein, Furchgott et al., Vincenzi and West among others. It has been amply demonstrated that acetylcholine depresses the pacemaker potential and that catecholamines accelerate its rise.

In the work to be reported we determined quantitatively the after-effects of various rates and durations of imposed drives, the effects of liberation of transmitter substances, and the consequences of artificial drive after their blockade. We also obtained evidence of conduction failures in the SA node and pacemaker shifts which were of considerable interest. Due to our previous work, we were able to make comparisons with imposed drive on in situ pacemaker tissues.

Methods

SA nodal tissues were obtained from 36 mature cats of either sex. Initially pentobarbital sodium (40 mg/kg) was employed but better results were obtained when hearts were removed under ether anesthesia. A strip of tissue containing the node and a small fragment of the right atrium (fig. 1) was dissected out and

![Diagram of sinoatrial node region as exposed. Strip of tissue (1 to 2, a to c) removed containing fragment of right atrium. RA: right atrial appendage. CT: crista terminalis. SA: upper sinoatrial node location of "true" pacemaker cells shown by large dots. SA': lower sinoatrial node. Broken line (b - - - - b') indicates division between areas considered "upper" and "lower" SA nodal regions. ST: position of driving electrode.](http://circres.ahajournals.org/)

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fixed in the tissue chamber of a perfusion bath in a fashion commonly employed for intracellular recording. Temperature was held at 37°C and a constant perfusion of oxygenated (O₂, 95% and CO₂, 5%) Tyrode solution was maintained at a flow rate of 2 ml/min. When drugs were administered they were dissolved in the replacing solution or added directly to the tissue chamber.

Silver bipolar electrodes placed on the surface of the tissue as shown in figure 1 were used for stimulation. Stimulating pulses, usually of 1 msec duration, were provided from a Tektronix unit consisting of a power supply, a waveform generator, a pulse generator, and were passed through an isolation unit. Driving stimuli were applied at intervals varying from 100 msec (rate of 600/min) to 630 msec (95/min). Drive durations varied between five seconds and five minutes.

Conventional Ling-Gerard microelectrodes were used for intracellular recording. Changes in membrane potential were monitored through a cathode-follower on a Tektronix 502 dual beam oscilloscope and photographed by a Grass kymograph camera. Surface recording and intracellular recording from two adjacent cells were also done but in most instances single cell responses were studied, one beam being used to record the entire action potential and the other to monitor the greatly amplified prepotential changes.

The cells which have the characteristics of “true” or dominating type of pacemaker cells were found to be in the upper regions of the node (fig. 1 SA) while cells in the lower regions of the node (fig. 1 SA') were “latent” or subsidiary pacemaker cells in that they depolarized more slowly and their spike potentials rose precipitously as though a result of propagated excitation. We found it useful to speak of the upper and lower SA node regions and the nature of this division is indicated in figure 1.

When comparisons were made between reactions of upper and lower SA nodal cells, the tissue strip was cut horizontally as shown (fig. 1 b-b'). Each preparation was allowed to equil-

![Figure 2](http://circres.ahajournals.org/)

**FIGURE 2**

Effects of severing connections between upper and lower SA nodal cells. A: lower nodal cell activity (upper tracings) before (A1) and after cutting connections (A2). Effects of driving at rates above the intrinsic (—) on upper nodal (lower tracings) and lower nodal cells (upper tracing of A2). B: lower nodal cells after separation from upper node. Pacemaker action in lower node was slow, irregular and readily depressed. In B upper tracing is a surface recording. Time 1000 msec; amplification 50 mv.

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ibrate for at least one hour in the tissue chamber before any tests were made. Intrinsic pacemaker rates were always determined before driving was attempted and the effects of the driving to be used were ascertained before drugs were administered. In repetitive testing adequate intervals for recovery were allowed before additional drives were imposed or drugs given.

In estimating the magnitude of the post-drive depressions produced, three calculations were made: time to the first post-drive beat, time required for the first ten or twenty beats, or time to recovery of a steady state (control rate). Other details of procedure will be given during description of results obtained.

Results

All sinoatrial node preparations containing upper nodal tissue continued to beat regularly after removal from the heart when perfused with warm oxygenated Tyrode solution. None of the lower SA nodal preparations maintained an intrinsic rhythm immediately after connections with upper nodal cells were severed. Some resumed beating spontaneously after quiescent periods varying from a few seconds to several minutes in duration. Others resumed automaticity only after external stimulation had been temporarily applied. Four of the five lower SA nodal tissue fragments studied beat at a rate significantly slower than did the upper part of the node and three of the five showed marked irregularities of rhythm (fig. 2B). Figure 2 (A1 and A2) shows the change produced in one sample experiment following a dissociation of upper and lower nodal pacemakers by severing connections between them.

As might be expected, cells with different type action potentials were found in the upper primary pacemaker region. Cells showing a diastolic depolarization (prepotentials) and repolarization "undershoot" (hyperpolarization) were considered to be pacemaker...
cells but only those with smooth transition from prepotential to spike potential and a slow rise time were considered to be true or dominant pacemaker cells. Most of these were located in the upper part of the SA node close to the endocardial surface and within 1 to 3 mm from the crista terminalis as shown by Paes de Carvalho et al.10 in the rabbit and by Miyauchi11 in the dog.

The ability of the various cell types to follow driving stimuli differed. Most preparations could follow a drive of 375 pulses/min. True pacemaker cells were less capable of following a fast drive than were nonpacemaker and latent or subsidiary pacemaker cells. Figure 3 shows that under a fast drive the amplitude of the action potentials of true pacemaker cells declines. The faster the drive the more rapid the decline. At very fast driving rates pacemaker cells responded minimally if at all. During the post-drive period spike amplitudes returned to normal as rate increased.

Another difference shown in figure 3 is that the durations of pacemaker cell potentials were not reduced as much as were those of nonpacemaker fibers. The first action potentials recorded in the post-drive period were actually longer than during the control states.

Pacemaker cells and most latent or subsidiary pacemaker cells showed some degree of hyperpolarization during imposed drive. This required three to five seconds to reach its maximum and it did not increase much as drive continued. The degree of hyperpolarization increased with rate of drive within limits. Hyperpolarization persisted for only a few seconds after termination of the drive and usually disappeared after a few beats (fig. 4).

It was found in studies of dog hearts in situ that pacemaker suppression is, within limits, proportional to rate and to duration of the drive.1 Figure 5 shows that suppression of pacemaker action increases with duration and also with rate of drive in both in situ and isolated SA nodal tissue of cats. Above 50% acceleration of rate, no additional or even less post-drive depression occurs. Whatever the depressing influence may be, there is an optimum rate of drive for its accumulation. Figure 6 gives the data obtained in 398 tests on 34 preparations in which the relation of percentage increase in drive rate to extent of post-drive depression was deter-

**Figure 4**

Effects of driving a sinoatrial nodal cell at a uniform fast rate (300/min or cycle length 200 msec) for five seconds (upper records) and ten seconds (lower records). Upper tracings are of same cell as in lower but at a higher amplification. Note hyperpolarization time courses and slightly longer post-drive depression after longer drive.
Comparison of depression in isolated (-----) and in situ (-----) SA nodal tissue of cats after drives of five and thirty seconds at rates of increasing percentage above control intrinsic rates. Per cent depression is per cent over time required for 20 beats at the control rate.

In production of post-drive depression the interval between beats is important. When a drive was imposed for 10 seconds, and when an interval of 400 msec was maintained between driving stimuli, the time required for ten post-drive beats increased by 25%. When the same number of stimuli were given in five seconds (200 msec intervals between stimuli) a greater post-drive depression resulted. Thus, the time to first beat increased by 120% and the time required for the first ten beats increased to 151% above the control values.

Figure 8 shows superimposed action potentials from a pacemaker cell. It can be seen that the rise time of the prepotential and the time to spike generation is greatly prolonged after drive. It also shows a reduction in amplitude of the intrinsic pacemaker potential. The spike generated in the first and third post-drive response propagated apparently from a different cell; the initial hump in the first tracing was probably the cell’s local response. Figure 9A shows an example of post-drive depression of the pacemaker potential, but in this case it was able to fire the cell. In subsequent beats this prepotential augmented gradually to normal amplitude. In some instances depression of pacemaker action in a cell by rapid drive re-
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sulted in permanent loss of dominance. As shown in figure 9B, although this dominant pacemaker cell's prepotential recovered progressively, it did not generate an action potential but firing was triggered from an adjacent cell. Eventually dominance by the initial pacemaker cell was restored.

Following rapid stimulation of SA nodal tissue, subthreshold oscillations of the resting potential occur (fig. 9C). It was found that the suppressing effect of rapid drive acted not only by reducing the amplitude of the pre-potential to ineffective values but also by reducing the rate of occurrence below that of the pre-drive state. This phenomenon appeared more frequently and persisted longer after an anticholinesterase was given. Epinephrine tended to hasten the return of these subthreshold potentials to normal intensity and restored a normal firing pattern more quickly.

In figure 9C, which shows action potentials from the same pacemaker cell at different amplifications, it can be seen that oscillations sometimes occur from a basic potential level somewhat lower than control values as though the membrane were unable either to initiate and propagate an action potential or repolarize to normal levels. It seems also that the threshold for initiation of a regenerative or propagated response had been raised (fig. 9C and D). Recovery in these instances involved return to a normal base line, growth of the local response to normal amplitude, a lowering to normal of the threshold for spike generation, and a regaining of dominance by this pacemaker cell. The first four post-drive propagated potentials of figure 9C obviously originated in a neighboring pacemaker, propagating with some decrement. Figure 9D shows the change in base line and thresholds for propagation but only one local response. Here, too, the initial action potentials were of ectopic origin indicating slower recovery of dominance by this pacemaker cell.

One of the most interesting findings of this work was the conduction block which occurs within the node. This phenomenon requires more study but multiple recordings showed that even under "normal" conditions block of propagation occurs between cells. After rapid driving this tendency for block to develop is augmented. Figure 10 shows a true pacemaker cell driving an adjacent subsidiary pacemaker cell. After a period of rapid drive, slow local responses occurred only in this pacemaker but periodically a distant pacemaker triggered a response in it. This action potential, however, did not propagate to the latent or subsidiary...

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Figure 6

Increase in post-drive depression with rates of drive at various percentages over the basal intrinsic rate. Time to first beat (A) and time required for first ten beats (B) expressed in percentage prolongation above normal. Standard error given; data from 34 preparations.

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Augmentation of depression, expressed in percentage increase of time to first beat and of time for first ten beats, as affected by prolonging the duration of drive. Note that acceleration occurred after five minute drive.

As recovery proceeded the impulse from the new ectopic pacemaker regularly reached the pacemaker and occasionally fired the second cell. As the original pacemaker regained control, conduction block also disappeared.

It is the opinion of pharmacologists that the suppression following drive is due, at least in part, to release of acetylcholine. Our results are in agreement with this because addition of Prostigmin to the perfusion bath augmented post-drive depression, increased hyperpolarization during drive and fostered conduction block. Atropine counteracted the depression (fig. 11). When atropine alone was given it reduced but did not abolish post-drive depression and, therefore, it was concluded that factors other than acetylcholine release and accumulation are involved.

One obviously possible effect of rapid drive is a change in membrane permeability to potassium, in durations of K⁺ ion flux and in potassium gradients. This matter requires more study but increasing external potassium concentration by 50 to 100% reduced post-drive depression.

Control
20th.
3rd.
1st.

Superimposed action potentials showing rise time and amplitude of prepotential at time of spike generation. See text.
We found as did West\(^3\) that post-drive depression ended in an overshoot or supernormal acceleration of pacemaker action. The latency, amplitude, and duration of this acceleration depended upon rate and duration of the drive. Faster rates of drive produced greater immediate depressions but also higher and longer lasting accelerations above control rates. The duration of depression was frequently shortened by the augmentation of acceleratory influences due to faster driving. Longer durations of drive augmented late acceleration as they did the early depression of pacemaker action. In some preparations the acceleratory post-drive effects outweighed depressor actions and, in these, longer duration of drive gave earlier, greater, and longer lasting accelerations up to a limit. Figure 12 illustrates this point, showing that after the longest drive both inhibition-producing and acceleration-producing influences were maximal. It has already been shown in figure 7 that driving at rates only slightly faster than normal (less than 20\% faster) tends to cause acceleration and little or no preceding depression. It was also shown in

**FIGURE 9**

A: depression of pacemaker potential by rapid drive and its post-drive recovery without loss of pacemaker action. B: depression of pacemaker potential with loss of dominance. C: local responses of subnormal frequency and a temporarily maintained post-drive hypopolarization and increase in threshold potential (C and D). See text.

**FIGURE 10**

Record of activity in a pacemaker cell (lower tracing) which, before imposed drive, initiated firing of an adjacent cell (upper tracing). Failure of local responses to evoke action potentials is shown. Action potentials originating in ectopic pacemakers failed, following drive, to propagate from one cell (original pacemaker) to its original subsidiary cell. See text.

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and cells which show little or no prepotential. Even under relatively normal conditions the site of origin of beats may shift somewhat within the node.13, 14 It is of interest that true pacemaker cells can be driven less rapidly than can the other types of nodal cell15 and that they recover from post-drive depression less rapidly. Thus, after drive, a shift of pacemaker is usually observed. Cells other than those from which recordings were made tended to initiate the first post-drive propagated responses. This slowness of response and recovery characterizes nodal cells of the pacemaker category.16

Certainly, electrical stimuli cause discharge of an admixture of acetylcholine and nor-
Changes in post-drive depression and acceleration as produced by driving at uniform rates for various periods of time. See text.

Epinephrine from nerve endings and tissue stores. The fact that post-drive depression is reduced but not abolished by atropine suggests that the effects may be, in part, the consequence of ion shifts and changes in membrane permeability not fully dependent on acetylcholine release. Acetylcholine is thought to act by increasing membrane permeability to potassium. The hyperpolarization during drive could be due to augmented K+ efflux; the slower rise and lower amplitude of the pacemaker potential and the occurrence of small local potentials after drive could be due to increased extracellular potassium or potassium efflux such as occurs during acetylcholine action and even in its absence. The post-drive rise in base line observed in sinus cells could indicate an abnormal potassium transmembrane balance. Even the acceleration following minimal rates of drive might be influenced by a K+ ion shift as well as by catecholamine release. It has been stated that the spontaneous rhythmicity of sinus fibers is
enhanced by an extracellular potassium concentration sufficiently above normal to cause partial depolarization of the cell. 19

The presence of acetylcholine and an excess of extracellular potassium might also explain the failure of conduction within the sinoatrial node following rapid drive. Studies of conduction within the atrioventricular node 10,11 revealed the marginal nature of the factor of safety for conduction in such tissue. It is not surprising to observe block and decrementing propagation in the sinoatrial node. Post-drive depression might be due, in part, to failure of conduction of activity from pacemaker cells as well as to suppression of activity within the pacemakers. The occurrence of both has been demonstrated in this work.

Our studies confirmed the work of West and others 4,6,9 who obtained evidence that catecholamine release occurs during drive of pacemaker tissues. We found that in certain preparations the post-drive acceleration response was more predominant than in others; it, like the depressor influences, showed a quantitative relationship to the rate and duration of imposed drive. Responses of isolated and in situ pacemaker cells to drive are much the same.1

Summary

Rapid drive of isolated pacemaker tissues from cats resulted in a post-drive depression followed by a late acceleration to supranormal rates of pacemaker activity. These effects were similar to those occurring after drive of the pacemaker in situ. Lower SA nodal pacemakers discharged more slowly and irregularly than did upper SA nodal pacemaker cells. They were more readily depressed by rapid imposed drive. The balance between depression and acceleration varied in different preparations. Drive at only slightly above the intrinsic rate resulted frequently in acceleration not preceded by depression. Within limits, the greater the frequency and duration of drive, the greater the intensity and duration of both the depression and the late acceleration.

Prostigmin augmented and atropine reduced post-drive depression. Cocaine potentiated the late acceleration. Excess potassium reduced post-drive depression and, in concentrations used, caused some acceleration.

Pacemaker cells could be driven less rapidly than could other SA nodal cells. Drive generally shifted pacemaker action to a distant site; the first post-drive propagated responses originated from other pacemaker cells and dominance by the original unit was reestablished slowly. Rapid drive reduced amplitudes of action potentials and prepotentials. It also raised threshold potentials and during the post-drive period the durations of pacemaker cell action potentials were temporarily prolonged. In some preparations membrane potentials remained at a subnormal value after drive. Subthreshold potentials occurred at a somewhat subnormal rhythm but gradually developed an effective amplitude.

Conduction block was observed in isolated SA nodal tissue. This was augmented during the post-drive period of depression.

This work lends support to the hypothesis 1 that dominating action by pacemaker cells influences the pacemaker activity in other potential pacemaker tissues.

References

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