Autorhythmic contractions of bat wing veins: Functional correlates between central and peripheral vascular pacemaker activity

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As the term 'venous heart' implies, the rhythmical movements of the venous vasculature in the patagial membrane of the bat wing are in many ways similar to the rhythmical contractions of the heart. It would appear that, just as in the heart, the aggregate of muscle cells enveloping the walls of these vessels are functionally highly organized, exhibiting the electrical properties of excitability, autorhythmicity and cell-to-cell conductivity. In recent years our understanding of these same properties in cardiac pacemaker cells has advanced considerably. This knowledge has come as the result of an intense effort to delineate the cellular basis for pacemaker activity using a variety of experimental techniques including microelectrode recording of transmembrane potential, ionic substitution, ion specific blocking agents, tissue culture analysis and most recently voltage clamp analysis. Investigations of rhythmical activity in wing veins, while not nearly as advanced as studies of cardiac automaticity, have provided a reasonably clear phenomenological description of rhythmical venous vasomotion. It is our purpose in this review to compare the behavior of these two autorhythmic systems with the hope of providing, by analogy, further insight into the cellular basis for electrical activation of the 'venous heart'.

Myogenic nature of pacemaker activity

Experiments designed to eliminate the possible influence of autonomic nerves have demonstrated that the initiation and distribution of the excitatory impulse in both the heart and in the bat wing vein are myogenic in nature. That is to say, the mechanisms responsible for such activity are inherent properties of the muscle cells involved. This does not imply, however, that the effect of the local milieu upon myogenic activity might not include the effects of nerve supply to that milieu. In fact in the case of the heart, it is quite clear that a major source of extrinsic modulation of pacemaker function is expressed through sympathetic and parasympathetic innervation of the sinoatrial node. While neurogenic regulation of venous vasomotion appears far less refined, evidence does suggest the possibility for some degree of control over the chronotropic and inotropic states of these vessels. It has been demonstrated, for example, that just as in the heart, the application of catecholamines can increase the frequency and amplitude of vasomotion in intact animals as well as in isolated veins.

Perhaps the most convincing evidence for the general phenomenon of myogenic autorhythmicity is that from studies of the developing heart showing that pacemaker activity precedes innervation. Furthermore, studies of heart cells in isolated fragments and in tissue culture have demonstrated that all portions of the premature heart contain cells with intrinsic pacemaker properties each beating at a different rate. Furthermore, it is important to note that pacemaker cells comprise only a small fraction of the total population of cardiac cells even during early development. As cells begin to aggregate, intimate cell-to-cell contacts form and eventually the entire aggregate is paced by that portion with the fastest spontaneous rate. While this process of entrainment accounts for the normal dominance of the 'fastest' pacemaker, it is also clear that the remaining pacemaker cells retain their automatic properties and under various conditions may initiate the heart beat. That similar conditions obtain at the level of the 'venous heart' is suggested by the observation that contraction waves may originate anywhere along the intervalvular segment indicating a distribution of cells with pacemaker capability. The preponderance of pulse wave propagation in a central direction suggests a dominant pacemaker located distally. As mentioned above, in order for a single locus to dominate the pacing function at a given moment, there must be electrical continuity among all cells forming the functionally integrated aggregate. The syncytial nature of cell-to-cell communication within the venous segments is indicated by the fact that the excitatory wave propagates at a rate 1 to 2 orders of magnitude faster than the peristaltic pressure wave. In other words, excitation precedes contraction making it unlikely that the propagated pressure pulse participates as a mechanical stimulus to advance the excitatory wave front. Direct evidence from experiments in which electrical and mechanical responses were recorded simultaneously demonstrates unequivocally that in rhythmically contracting veins just as in the heart the excitatory pulse precedes contraction. It is noteworthy in this regard to point out that conduction velocities reported for venous segments are comparable to those reported for the sinoatrial and atrio-ventricular nodes (0.02–0.05 $M \cdot sec^{-1}$) suggesting a similar mode of cell-to-cell transmission.

Microelectrode measurements of membrane potential have established basic criteria for identifying pacemaker and non-pacemaker cells wherever they may occur. By definition pacemaker cells can depolarize spontaneously and non-pacemakers cannot. Nicoll

and Speden, using intracellular recording techniques, have demonstrated the presence of cells within patagial veins that depolarize spontaneously with a gradual transition between the pacemaker prepotential and the upstroke of the ensuing action potential, i.e., the *sine qua non* for true pacemaker activity. Working in collaboration with H.-J. Huggel, we have confirmed these results in our own laboratory using pteropid metacarpal veins and have noted that such cells appear to be distributed along the entire intervalvular segment.

To summarize the points made thus far, it would appear that rhythmically contracting veins, like the heart, are functionally comprised of syncytially organized contractile cells of which a small fraction are capable of initiating the self-propagated excitatory impulse. Within this syncytial array the fastest pacemaker dominates. Kallen has pointed out that this may be of particular importance in veins comprised of large, multilayer muscle masses where a specific pacemaker locus may be necessary to coordinate and synchronize the rhythmical activity as is the case in the heart. Experimentally, such a locus would not be expected to be demonstrable with averaging techniques such as sucrose-gap. At more peripheral sites, however, where vessels contain only a single layer of smooth muscle, it might be expected that less sophisticated organization is required and that multiple pacemaker activity may obtain.

Hierarchical control of pacemaker function does not preclude the possibility for shifts in pacemaker location or for competition among pacemakers for dominancy. Such well known cardiac arrhythmic phenomena as 'wandering pacemaker' and 'ectopic extrasystole' may well have parallels in the normal function of the 'venous heart'.

Importance of mechanical stretch

One of the most striking functional similarities between the sinoatrial node and rhythmically contracting veins is their response to mechanical stretch. It is now well established that both tissues respond to stretch by increasing their spontaneous beating frequency. This positive chronotropic response does not depend in either case on neurogenic factors but rather appears to depend on the direct action of stretch to deform the pacemaker cell membrane. In the case of sinoatrial node pacemaker cells, it has been shown that stretch causes generalized depolarization without any change in threshold potential. This alone could account for the acceleration of spontaneous rate. Moreover, an increase in extracellular calcium enhances stretch-induced acceleration. Although the ionic basis for the stretch response remains uncertain, it is known to be associated with a decrease in membrane resistance. This fact, coupled with the above-mentioned action of extracellular calcium, suggests that a change in calcium permeability may play an important role in the response to stretch. Whether or not these same factors might contribute to the frequency response in patagial veins in a similar or identical way is at present unknown.

It has often been suggested that a certain degree of stretch is prerequisite to spontaneous activity in contracting veins and that for this reason venous automaticity is mechanistically different from that of the sinoatrial node. We believe that this distinction is unwarranted for at least 2 reasons: Firstly, it has been shown that in an initially quiescent sinoatrial pacemaker cell the application of stretch causes a gradual decrease in resting membrane potential which leads eventually to the generation of rhythmically occurring action potentials. This situation is presumably analogous to pressure-induced automaticity in a quiescent venous segment. Secondly, spontaneous activity, albeit very erratic, can occur in venous segments exposed to zero internal pressure. In other words, pacemaker cells in both sites are capable of spontaneous activity in the absence of 'apparent' stretch, and yet under conditions when the pacemakers are initially quiescent mechanical stretch will initiate and accelerate spontaneous activity.

The fact that sinoatrial node pacemaker cells have a greater predisposition for spontaneous discharge may depend on the unique arrangement of parenchyma and stroma within the node. James suggested that nodal cells are under constant tension due to their attachment to collagen fibres which form a network attached to the centrally located sinus node artery. This relationship is such that a decrease in the diameter of the artery, as might be expected with reduced arterial blood pressure, increases tension in the collagen fibres, which further stretches the nodal cells and causes tachycardia. It is further postulated that through this mechanism pulsatile arterial pressure may act as a servomechanism for stabilizing the sinus rhythm. Although an analogous relationship of stroma to parenchyma has not been described for patagial veins, the important point we wish to emphasize is simply that in both tissues mechanical stretch expressed through changes in transmural pressure may play an important physiological role in modulating pacemaker function.

In addition to the modulation of spontaneous rate, stretch may also serve to regularize the rhythm of contraction. It has been reported, for example, that the application of stretch to irregularly beating sinus node preparations will result in a regular rhythm associated with a slight decrease in maximum diastolic potential. The results obtained from multiple electrode recordings suggest that this improvement of rhythm may be due to an improvement in intercellular conduction. That such a mechanism might in fact

operate in venous segments is suggested by the observation that at higher luminal pressures the rhythm of venous contractions is typically more regular.

Ionic basis for venous automaticity

As indicated earlier, microelectrode studies have revealed that in both sinus node and venous pacemaker cells spontaneous depolarization of resting potential eventually reaches threshold leading to the formation of an action potential. Experiments designed to examine the role of potassium, sodium and calcium in this process have yielded the following salient facts: 1. Compared to other cardiac cells, sinus node and venous cells are relatively resistant to the depolarizing effects of elevated extracellular potassium [K^+_o]. These cells do depolarize when subjected to high K^+_o but not nearly to the extent expected of cells whose resting potentials are determined largely by the potassium equilibrium potential. 2. Increases in K^+_o within limits accelerate the spontaneous discharge of both sinus node and venous pacemaker cells. Excessive potassium depolarization renders these tissues quiescent and inexcitable. Such acceleration is in striking contrast to potassium effects on ventricular pacemaker cells, i.e., Purkinje fibres. In these cells high K^+_o has a suppressive action on automaticity most likely due to an increase in potassium permeability caused by potassium itself. 3. The reduction or removal of extracellular sodium either has no effect or causes a slight increase in the firing frequency of both sinus node and venous pacemakers. The lack of participation by sodium in this process in the sinus node has been further demonstrated using tetrodotoxin (TTX), an agent that blocks both background and excitatory sodium current in the heart. In concentrations sufficient to prevent the formation of action potentials in other heart cells, TTX is without effect on the sinus node. To our knowledge TTX has not as yet been applied to patagial veins. On the other hand, reduction of Na in the perfusate is known to decrease the firing rate of Purkinje fibres by decreasing the rate of diastolic depolarization. 4. An increase in extracellular calcium within limits accelerates both sinus node and venous pacemakers cells. Conversely, the complete removal of Ca^{++} using EGTA causes quiescence. This again contrasts with the response of Purkinje fibres where automaticity is suppressed by calcium due to a shift of threshold potential to less negative values. 5. Verapamil, an agent with specific blocking effects on slow inward calcium current, completely suppresses sinus node and venous automaticity.

Taken collectively these observations are in strong support of the concept that the ionic basis for pacemaker activity in the venous heart is qualitatively similar to that found in the so-called true pacemaker of the sinus node. Furthermore, it is clear that substantial differences exist between these primary pacemaker cells and subsidiary pacemakers represented in this comparison by ventricular Purkinje fibres.

These findings, along with recent voltage clamp data from sinus node cells, provide the basis for a plausible explanation of venous automaticity. At rest, membrane potential in the venous pacemaker cell most likely depends on the outward flow of potassium current and a substantial background inward current probably carried by calcium. In other words, there is a relatively low potassium-to-calcium conductance ratio which would explain both the low value of resting potential and the relative resistance of these cells to the depolarizing action of high K^+_o. In the presence of background calcium current a time-dependent decay in outward potassium current would give rise to the pacemaker prepotential necessary to reach threshold. The firing rate would thus depend on the rate of decay of outward current and the magnitude of the inward background calcium current. The observed dependence of spontaneous rate on extracellular calcium might well be explained in this manner. In fact in sinus node cells, it has been shown that the positive chronotropic action of elevated extracellular calcium is indeed due to an increase in the rate of diastolic depolarization. As threshold is reached the voltage-dependent opening of slow inward channels allows for the influx of calcium, and perhaps sodium, leading to the formation of a slow rising, low amplitude action potential. The persistence of this activity in the absence of extracellular sodium suggests that the development of the pacemaker prepotential and spike electrogenesis in these cells are both calcium-dependent. The action of verapamil to block venous vasomotion further supports this notion.

In summary, the paucity of direct data from venous pacemaker cells precludes a quantitatively more satisfying description of venous automaticity. The tentative explanation offered here, albeit speculative, is consistent with existing experimental findings and with our current understanding of sinus node automaticity. The extent to which our speculations are valid for this system, and the extent to which they may apply to analogous systems, must be determined through further experimentation.

For detailed review of material covered in this article, see the following:
1 C.McC. Brooks and H.H. Lu, The Sinoatrial Pacemaker of the Heart. Charles C. Thomas, Springfield, Illinois, 1972.
2 T.N. James, Henry Ford Hosp. med. Bull. *15*, 275 (1967).
3 F.C. Kallen, Biology of Bats, vol. III, chapter 3. Academic Press, New York 1977.
4 D. Noble, The Initiation of the Heartbeat. Clarendon Press, Oxford 1975.

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