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Cardiac Pacemaking: An Obligatory Role of Catecholamines?

A possible mechanism underlying spontaneous pacemaking can be deduced from several recent clues.

Gerald H. Pollack

In the most recent comprehensive review of sinus node function, Brooks and Lu (*1*) concluded that the underlying mechanism of pacemaker action in the heart remained a mystery. Since then, several clues have been found which make it appropriate to reexamine the question of pacemaking in a new light. It appears that a solution to the mystery may lie in a heretofore unrecognized role of catecholamines.

It is well known that catecholamines increase the speed of spontaneous depolarization in pacemaker cells, thereby elevating the heart rate (*2*). It now appears that this familiar postsynaptic role of catecholamines may be complemented by an equally important presynaptic role. In this article I consider the evidence that pacemaker cells store and secrete catecholamines, and that they are unable to pace when deprived of the action of both pre- and postsynaptic pools of catecholamines.

Do Pacemaker Cells Store Catecholamines?

Early histological studies of the heart demonstrated that the region of the sinus node was densely innervated (*3*). Thus, when the paraformaldehyde fluorescence technique was applied to evaluate the regional distribution of catecholamines in the heart (*4*) it came as no surprise that the sinus node was richly endowed with catecholamines. However, not all the catecholamines are stored in nerve terminals. In dog hearts that had been denervated both surgically and chemically (with 6-hydroxydopamine), the sinus node was found to have retained about half the epinephrine present in nondenervated controls (*5*). The authors of this study concluded that the storage of catecholamines was in part extraneural, possibly in specialized catecholamine-storing cells which were analogs of the chromaffin cells of the adrenal medulla.

Extraneural storage of catecholamines is also found in the embryonic heart. Although embryonic heart cells and pace-

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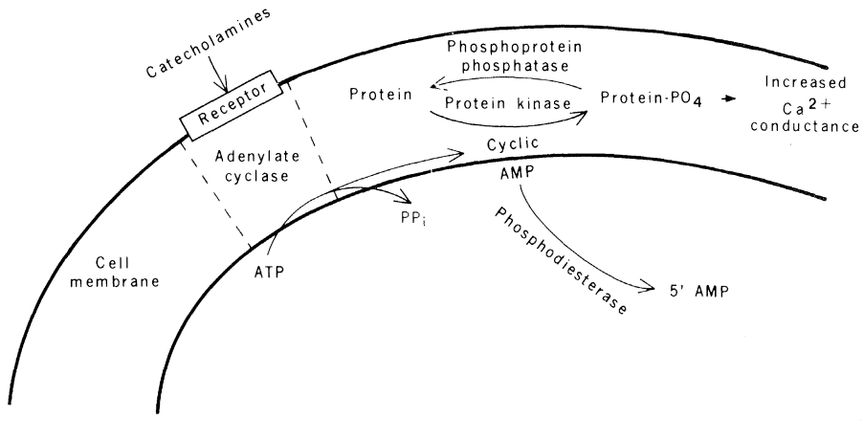

Fig. 1. The mechanism by which catecholamines appear to increase calcium influx into the cell (PP_i, pyrophosphate). [After Greengard (*27*)]

maker cells are not identical, both have primitive, relatively undifferentiated cellular structures, an observation which has contributed to the widely held view that pacemaker cells are developmental remnants of embryonic heart cells (*6*). Adrenergic innervation does not occur in the embryonic chick heart until day 16 of development (*7*); yet catecholamines are found in the beating heart by day 3 (*8*). A similar temporal disparity was found in embryonic hearts of humans (*9*). In this last-mentioned study, the catecholamines were localized in intensely fluorescent cells which, by the fourth month, had appeared in numerous clusters in the region around the interatrial septum. When cells from embryonic hearts were dispersed, cultured, and allowed to beat spontaneously in a nerve-free environment, they were found to store catecholamines (*10*).

The ultrastructure of pacemaker cells seems consistent with the possibility of catecholamine storage. Electron micrographs of cells in the sinus node show rather uniformly sized, subsarcolemmal vesicles (*11–14*). In one report (*13*) the vesicles were observed to be "conspicuously numerous." In another (*11*) special note was also accorded these structures. The authors of both reports commented on the structural similarity of these vesicles to those found in the chromaffin cells of the adrenal medulla, and suggested that sinus node cells, likewise, probably store catecholamines.

The possibility of subsarcolemmal storage of catecholamines is consistent with the fluorescence histochemistry. According to the patterns recently published (*15*), catecholamines are distributed in the sinus node in a mosaic-like array, with somewhat irregular 10- to 15-μm oval-shaped units forming a contiguous, very bright meshwork. While this pattern is grossly inconsistent with the distribution of nerve terminals (*16*), it is consistent with catecholamine disposition in subsarcolemmal regions of each pacemaker cell. On the other hand, the possibility that the fluorescence is derived from an extracellular connective tissue matrix has not been excluded.

Is Pacemaking Possible Without Endogenous Catecholamines?

Tuganowski *et al.* (*17*) exposed isolated rabbit sinoatrial node to reserpine, an agent which depletes catecholamine stores by inhibiting uptake into vesicles. Reserpine (10 μM) slowed the spontaneous rate and brought beating to a halt in 5 to 25 minutes. When catecholamines were added to the bath containing the reserpine, within 1 to 3 minutes beating resumed. In another series of experiments the node was exposed to α-methyl tyrosine (1 mM), a specific inhibitor of catecholamine synthesis. Spontaneous activity stopped in 1 to 4 hours. When catecholamines were added to this bath, beating resumed in 2 to 5 minutes. When preparations were first treated with reserpine to deplete catecholamine stores, and washed with fresh Tryode's solution to restore beating, the time required for α-methyl tyrosine to inhibit beating was reduced 100-fold. The authors concluded that endogenously synthesized catecholamine stores were indispensable for normal spontaneous activity of the sinus node.

Similar results were found by Noda and Yugari (*18*) in spontaneously beating cultured heart cells. Beating ceased at concentrations of reserpine ranging between 2 and 20 μM; cells resumed beating on exposure to epinephrine or to norepinephrine, the response latency depending on the concentration. Beating was also restored by dopamine, a precursor of these catecholamines. When untreated cells had finally exhausted their ability to beat after several days in culture, beating could be restored almost to control rates within 5 minutes by exposure to catecholamines. The authors concluded that without endogenous catecholamines, or substitute stores supplied exogenously, cultured cardiocytes cannot beat.

An apparently obligatory role of catecholamines is also found in hearts of cyclostomes, among the most primitive of vertebrates. Lamprey hearts are only sparsely innervated, while hagfish hearts appear to be aneural (*19*); yet catecholamine concentrations in these hearts approach the levels found in the chromaffin cells (*20*). These remarkably concentrated catecholamines (130 μg/g in lamprey atrium) are found in specialized subendocardial cells, cells which were shown to have sufficient morphologic and biochemical similarity to chromaffin cells to prompt the suggestion that they might play a secretory role (*21*). Reserpine (0.1 to 0.2 mg, subcutaneously) slowed these hearts, bringing some to standstill; at the same time catecholamine levels were demonstrably depleted (*21*). Like the sinus node cells and cultured heart cells, beating was restored on exposure to catecholamines.

A Possible Role of Endogenous Catecholamines

Although the storage site of catecholamines is within the cell, the action of catecholamines on pacemaker (and other) cells is on the outside of the membrane (*22, 23*). If endogenous catecholamines play an obligatory role in beating, a way must be identified by which the catecholamines might leave the cell. One possibility is that the catecholamines are secreted from the cell, and that they then feed back upon the outside of the membrane. Although there is no direct evidence for this, electron micrographs of sinus node cells show numerous exocytotic figures (*13*). The correlation between the appearance of exocytotic figures and the process of neurosecretory discharge is so prevalent throughout the neuroendocrine system that the supposition of exocytotic discharge from sinus node cells does not seem unreasonable.

Signs of possible exocytotic discharge are also seen in developing mouse heart cells. In freeze-etch replicas of the membrane surface of these cells, Ishikawa and Yamada (*24*) found the membrane to be covered with numerous "pits" and "bumps." These structures were similar in size to the vesicles in the cells of the

sinus node. Although these have been presumed to be precursors of T tubules, they occur in numbers which are orders of magnitude higher than the ultimate number of T-tubular openings and, unlike T tubules, they are not regularly arrayed along the surface of the membrane. One wonders if these structures might not represent an even more vivid example of vesicles fixed in the process of exocytotic discharge.

Exocytosis, therefore, appears to be a feasible route by which endogenous catecholamines might reach the exterior of the membrane to mediate their action. What is their action? Some clues may be obtained by examining the action of exogenous catecholamines, since both endogenous and exogenous catecholamines are likely to share a common pathway of action.

One of the most fundamental actions of exogenous catecholamines is to increase the calcium flux into the cell (*25*). This might occur in one of two ways (*26*). Catecholamines might act directly on the outside of the membrane to open calcium channels. The other possibility, supported by a larger body of evidence, is that catecholamines increase the concentrations of intracellular adenosine 3′,5′-monophosphate (cyclic AMP) through activation of membrane-bound adenylate cyclase (Fig. 1); cyclic AMP then activates a protein kinase, which catalyzes the phosphorylation of membrane proteins and opens calcium channels, allowing calcium to enter the cell (*27*).

While catecholamine action causes calcium entry into the cell, calcium entry causes catecholamines to leave the cell. Calcium influx appears to be a necessary condition for exocytosis in perhaps all neurosecretory systems studied so far (*28*). The reason for such a requirement is unclear; one recent proposal is that the entering calcium ions bind to the negatively charged surfaces of the interior of the cell membrane and the exterior of the vesicle membrane, thereby diminishing the potential barrier between vesicle and membrane and increasing the probability of fusion and exocytosis (*29*). Whatever the actual mechanism, it is clear that Ca^{2+} influx depolarizes the cell and causes exocytosis. In the neuromuscular junction, perhaps the best studied secretory system, the rate of discharge varies exponentially with the degree of cellular depolarization (*30*).

In view of the fact that catecholamine action on the outside of the membrane causes Ca^{2+} influx, and Ca^{2+} influx can bring about catecholamine efflux, the elements of a regenerative feedback loop are at hand. Spontaneous depolarization and repolarization could then occur by the following scheme (Fig. 2A):

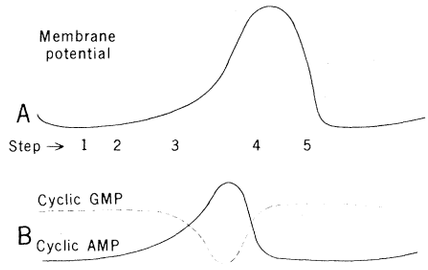

Fig. 2. (A) The time course of the pacemaker potential. The cycle is broken into five phases corresponding to events described in the text. (B) The time course of the concentration of intracellular cyclic AMP expected from the proposed model. Reciprocal fluctuation of cyclic GMP may also occur, as it does in another cardiac tissue.

1) Vesicles begin discharging catecholamines into the extracellular space; the discharge rate is low at first because the cell is well polarized (−50 to −60 mv) and the intracellular Ca^{2+} concentration is relatively low.

2) Catecholamines discharged into the extracellular space diffuse away from the discharge site; those molecules which bind to the outside of the cell membrane activate adenylate cyclase, thereby increasing cyclic AMP, bringing about the phosphorylation of membrane proteins, and transiently opening calcium channels; this allows Ca^{2+} to enter and further depolarize the cell.

3) The more the cell is depolarized by Ca^{2+} entry, the higher the rate of catecholamine discharge; this positive feedback loop results in a depolarization which has the shape of a rising exponential; ultimately all catecholamines in the releasable pool are discharged.

4) With no further catecholamine-mediated adenylate cyclase activity, cyclic AMP returns to baseline levels and there is no further opening of calcium channels; thus the cell stops depolarizing.

5) As the cell repolarizes by Ca^{2+} extrusion, the vesicles reform and are replenished with catecholamines; when replenishment is adequate, spontaneous discharge commences and the cycle begins once again.

This scheme describes a complete pacemaking cycle. Steps 1, 2, and 3 follow logically from the evidence already presented, while 4 and 5 are the simplest additional steps required to complete the cycle.

An important implication of this scheme is that the concentration of cyclic AMP is expected to oscillate during the pacemaking cycle, as illustrated in Fig. 2B. During steps 1 and 2 and most of step 3 the level of cyclic AMP increases, since catecholamine-mediated adenylate cyclase activity increases. When further catecholamine binding ceases, cyclic AMP production is no longer stimulated. Phosphodiesterase activity then breaks down cyclic AMP and causes a diminution of the pool. Baseline levels of cyclic AMP are reached during step 4, and with no further adenylate cyclase activity, cyclic AMP remains at this level until the cycle begins again.

Although the concentrations of cyclic AMP during the cardiac cycle have not yet been measured in pacemaker tissue, they have been found to oscillate in frog ventricular tissue (*31, 32*). In these studies the level of cyclic AMP increased in the early part of the cycle, returned approximately to baseline levels prior to repolarization and remained there until the beginning of the following cycle, a time course similar to the one in the proposed scheme (Fig. 2B).

Maintenance of stable cycling in this (or any) scheme requires that the processes mediating depolarization be prevented from occurring during the phase of repolarization; thus Ca^{2+} influx must not be allowed to occur beyond step 3. While this can be achieved in the scheme through phosphodiesterase-mediated diminution of the cyclic AMP level, two additional factors may augment this mechanism and thereby enhance cycling stability.

The first is the acceleration of phosphodiesterase activity during step 3. The relatively low activity found at low Ca^{2+} concentrations increases when the Ca^{2+} concentration is elevated above 1 μM to a greatly enhanced activity at 10 μM (*33*). Because there is good reason (though no conclusive evidence) to believe that intracellular Ca^{2+} rises to these concentrations at the peak of the cardiac cycle (*34*), it is possible that a greatly enhanced phosphodiesterase activity in step 3 may drive cyclic AMP down to baseline levels rapidly.

The second factor may involve guanosine 3′,5′-monophosphate (cyclic GMP), the other important cyclic nucleotide found in the cardiac cell. In accordance with the well-known "yin-yang" hypothesis (*35*), cyclic GMP antagonizes the action of cyclic AMP, probably by dephosphorylating proteins through activation of phosphoprotein phosphatase (*36*). The intracellular concentration of cyclic GMP oscillates during the cardiac cycle of frog ventricle with a time course similar, but a direction opposite, to that of cyclic AMP (*32*); this may occur through the conversion of one cyclic nucleotide into the other (*37*). Should cyclic GMP and cyclic

AMP fluctuate reciprocally in pacemaker cells as well (Fig. 2B), the effect of the cyclic GMP fluctuations would be to enhance phosphorylation at the time in the cycle that the processes mediating Ca^{2+} influx required activation (steps 1 to 3), and to prevent phosphorylation at the time these processes required inhibition (steps 4 and 5). Oscillations of cyclic GMP, if they occurred, would therefore serve to increase cycling stability.

Elements of the pacemaking cycle which are least clear are those occurring in step 5. Repolarization might occur by a delayed efflux of K^+ as it does in many other cells; a K^+-Ca^{2+} pump would then be required to maintain steady-state concentrations of these ions. A simpler possibility is that repolarization occurs as a direct consequence of Ca^{2+} being pumped out of the cytosol. A Ca^{2+}-dependent adenosine triphosphatase is known to exist in the membrane of the catecholamine vesicles of chromaffin cells (*38*), and an analogous one might exist in vesicles of the sinus node. If the process of exocytosis involves a continual turnover of vesicle membrane and plasma membrane (*39*), then a Ca^{2+}-dependent adenosine triphosphatase should also exist in the cell membrane; this could mediate Ca^{2+} extrusion from the cell. This area requires further study.

Another major question is whether or not the processes of vesicle reformation and refilling could occur rapidly enough to be consistent with the proposed scheme. The first detailed studies on vesicle-filling time have recently been published; in a giant synapse of the hatchetfish, vesicles are replenished in 200 to 500 milliseconds (*40*). Whether such a time scale might carry over to pacemaking cells is an open question.

To recapitulate, the proposed scheme accounts for the obligatory role of catecholamines in pacemaking. When endogenous stores are depleted, the processes giving rise to cellular depolarization are curtailed, and pacemaking ceases. Exogenous catecholamines can restore beating, or under normal circumstances augment the frequency of beating, by supplementing endogenous stores; catecholamines derived from both sources act through a common pathway. Thus the regulatory role of sympathetically discharged or circulating catecholamines is an inherent feature of this hypothesis.

Another natural feature is the regulatory role of acetylcholine. By activation of guanylate cyclase, acetylcholine increases cyclic GMP (*41*). This increase should diminish the rate of protein phosphorylation, giving rise to a diminished rate of Ca^{2+} entry, and consequently a diminished frequency of discharge. Acetylcholine diminishes the "secondary inward current" in frog sinus (*42*), an observation consistent with reduced rate of Ca^{2+} entry. It is evident that high enough concentrations could diminish the rate of protein phosphorylation sufficiently to account for the observed abolition of pacing (*43*).

Finally, since Na^+, K^+, and Cl^- play no major roles in the scheme, modest alterations of the extracellular concentrations of these ions ought not to affect cycling. In the sinus node, alterations of extracellular Na^+, K^+, and Cl^- to concentrations which have marked effects on nonpacemaker tissues of the heart are almost without effect on pacemaker cycling (*1, 43, 44*). Tetrodotoxin is similarly without effect (*45*).

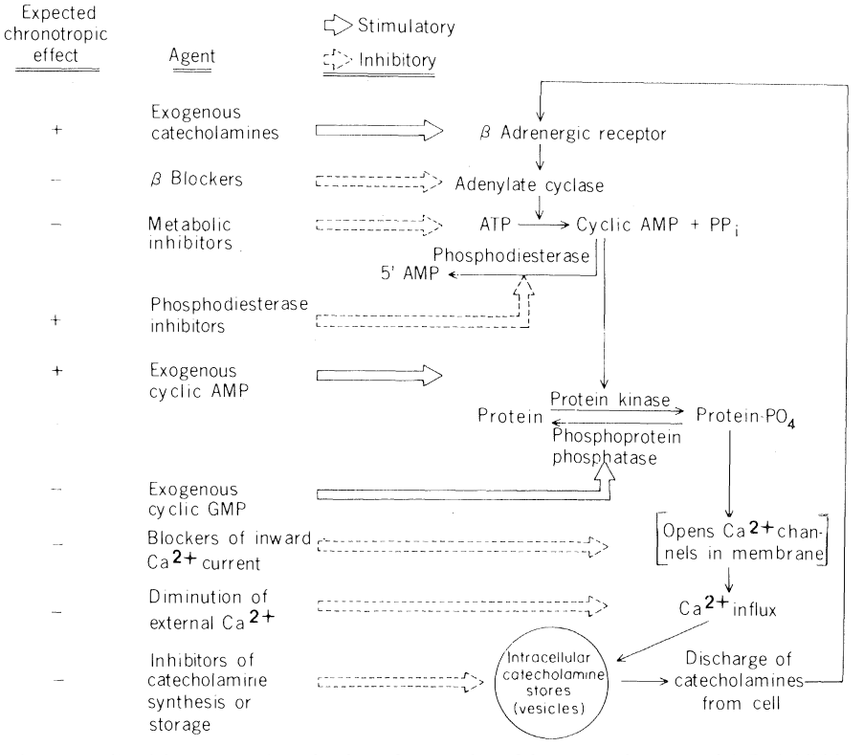

Fig. 3. Details of the proposed mechanism of generation of the pacemaker potential. By inhibiting or accelerating certain processes, the agents listed at the left of the figure should give rise to increases or decreases of pacing frequency. The expected responses are confirmed experimentally.

Evidence Bearing on the Proposed Role of Catecholamines

In the proposed scheme, the β-adrenergic pathway through which catecholamines mediate their action (Fig. 3) is assumed to play the central role in pacemaking. Since many steps along this pathway are known to be inhibited or accelerated by certain agents, a test of the proposed scheme can be made by examining whether each agent produces the expected chronotropic effect. The classes of agents in question (listed at the left in Fig. 3) are as follows.

Exogenous catecholamines. Although their positive chronotropic action requires little further discussion, one subtle feature of their role in this scheme is that their action is additive, not multiplicative, since they supplement endogenously discharged catecholamines. Cells containing an endogenous store which is inadequate to sustain beating should commence spontaneous activity on exposure to an adequate supply of exogenous catecholamines. This feature may explain the observation that the number of beating cells in heart cultures is increased substantially when catecholamines are added to the medium (*46, 47*).

β-Adrenergic blocking agents. At sufficiently high concentrations, agents that block β-adrenergic activity (including propanolol, pronethal, MJ-1999, methoxamine, *N*-isopropylmethoxamine, and dichloroisoproterenol) have negative chronotropic effects on mammalian sinus node (*48*). In cultured heart cells, $10^{-5}M$ propanolol has a negative chronotropic effect which is modest in the neonatal mouse (*47*), but which is potent enough in the rat to cause immediate ces-

sation of spontaneous activity (*18*). These negative chronotropic effects of beta blocking agents are generally classified as direct depressant effects on the cell; here a mechanism for these "side effects" is reconciled with the physiology of the cell.

Metabolic inhibitors. Unlike the well-known ability of axons to conduct action potentials for protracted lengths of time following depletion of adenosine triphosphate (ATP), maintenance of spontaneous electrical activity in the proposed scheme is directly dependent upon the availability of ATP. This follows because the processes mediating spontaneous activity, not the least of which is the creation of cyclic AMP, depend directly upon the pool of ATP. When oxidative phosphorylation was uncoupled by 2,4-dinitrophenol ($10^{-4}M$) in isolated sinus node, the rate of diastolic depolarization decreased (after a transient tachycardia), resulting in a diminished spontaneous rate (*49*). Exogenous ATP ($10^{-4}M$) added to the bath containing the inhibitor partially reversed the negative chronotropic effect.

When cells isolated from hearts of neonatal rats were exposed to 2-deoxyglucose, a glucose analog which inhibits glycolysis, a reduction of cellular ATP was accompanied by a decrease of spontaneous beating rate which was reversed by exogenous ATP (*50*).

When various combinations of inhibitors of glycolysis and oxidative phosphorylation were used to diminish ATP concentrations in cultured rat heart cells, minor reductions of ATP—sometimes by only 10 percent—were sufficient to inhibit spontaneous activity (*51*). Yet depletion of ATP by 85 to 90 percent of control levels was required before the electrically stimulated contractile responses were inhibited. These authors concluded that spontaneous rhythmic activity requires the maintenance of high steady-state concentrations of ATP.

Phosphodiesterase inhibitors. By increasing the concentration of cyclic AMP, phosphodiesterase inhibitors should increase the speed of depolarization and thereby elevate the cycling frequency. Dose-dependent positive chronotropic effects were observed on exposure of fetal mouse hearts in organ culture to theophylline (*52*), and on exposure of spontaneously beating right atria isolated from hearts of rabbits and guinea pigs (*53*) to both theophylline and caffeine. Theophylline provoked only a small increase of beating frequency in rat heart cells in monolayer culture (*54*); the limited response in this preparation was attributed to a theophylline-induced increase of cyclic GMP, which was almost as great as the increase of cyclic AMP. Phosphodiesterase inhibitors also increase the beating rate in molluscan hearts (*55*) and insect hearts (*56*).

Exogenous cyclic AMP. Although extracellular administration of cyclic AMP induces a positive chronotropic response in the hearts of lower phyla (*55, 56*), no response is found in mammalian hearts (*22, 54, 57*). The absence of effect has generally been attributed to the relatively low permeability of the cell membrane to cyclic AMP (*58*), and to the high phosphodiesterase activity of the cells. Thus Yamasaki *et al.* (*22*) did find a positive chronotropic response to cyclic AMP when, in the presence of theophylline, it was injected through microelectrodes into cells of the rabbit sinus node.

Dibutyryl cyclic AMP, a derivative of cyclic AMP which is both permeable to the cell membrane (*59*) and resistant to phosphodiesterase breakdown (*60*), has also been utilized to study the action of cyclic AMP. Although extracellular application of dibutyryl cyclic AMP appears to be without effect on fetal mouse heart (*52*), it produces marked chronotropic effects on cultured rat heart cells (*57*), on perfused rat and guinea pig hearts (*61*), and on isolated rabbit sinus node (*22*). In isolated sinus node preparations previously arrested with reserpine or α-methyl tyrosine, dibutyryl cyclic AMP had a resuscitative action (*17*): a majority of preparations resumed spontaneous activity when the dibutyryl cyclic AMP was added directly to the perfusate containing the inhibitors. This observation provides important evidence for the obligatory role of the β-adrenergic pathway in pacemaking.

Finally, by exposing cultured rat heart cells to cardioactive agents which either raise or lower the concentration of cyclic AMP and observing that the rate was directly proportional to the resulting cyclic AMP level, Ghanbari and McCarl (*54*) concluded that the role of cyclic AMP in the pacemaking process is probably a direct one.

Cyclic GMP. Negative chronotropic effects of cyclic GMP and dibutyryl cyclic GMP have been demonstrated on rat heart cells in culture (*54, 62*).

Blocking agents of slow inward current. By preventing the influx of Ca^{2+} these agents should reduce the rate of spontaneous depolarization, thereby diminishing the frequency of cycling. Verapamil, D-600, and Mn^{2+} applied to isolated rabbit sinus node do, indeed, bring about such effects (*63, 64*).

Reduction of external Ca^{2+}. This also should tend to diminish inward Ca^{2+} current, thereby diminishing the speed of diastolic depolarization. A dramatic effect is not necessarily expected, because external Ca^{2+} is normally abundant and not necessarily an important limiting factor. Modest reductions of external Ca^{2+} do bring about some reduction in the rate of spontaneous depolarization, both in rabbit sinus node (*43, 64, 65*) and in cultures of rat heart cells (*66*); these effects are more pronounced as Ca^{2+} is further reduced. The cultured cells stop beating when external Ca^{2+} is reduced to $10^{-5}M$; in the presence of noradrenaline ($10^{-5}M$), however, it is noteworthy that cessation of beating requires further reduction of external Ca^{2+} by two orders of magnitude.

Agents inhibiting catecholamine synthesis and storage. The effects of reserpine and α-methyl tyrosine on the reduction of heart rate have already been considered.

It is evident that the observed responses are concordant with the expected responses at each step in Fig. 3. The β-adrenergic pathway, therefore, appears to be an obligatory link in the process of pacemaking. The scheme detailed here merely identifies a positive feedback loop through which regenerative activation and deactivation of this pathway might lead to pacemaking. While this scheme is not necessarily unique, it provides a testable working hypothesis.

Elements of the scheme may be relevant in other tissues in which both the β-adrenergic pathway and some regenerative phenomena are implicated. In the myocardium, one example which comes immediately to mind is the regenerative release of Ca^{2+} from the sarcoplasmic reticulum, a process which is incompletely understood. Cardiac sarcoplasmic reticulum contains endogenous adenylate cyclase activity (*67*). Catecholamines are taken up by cardiac cells (*68, 69*), and at least some fraction of these catecholamines is disposed in the area of the myofilaments (*70*), and is therefore accessible to the sarcoplasmic reticulum. Agents which are known to trigger regenerative efflux of Ca^{2+} from stores within the sarcoplasmic reticulum are: a small amount of Ca^{2+} (*71, 72*), cyclic AMP (*73*), and the phosphodiesterase inhibitors, caffeine (*72*) and theophylline (*74*), the same agents that cause Ca^{2+} influx into pacemaking cells. It may be no coincidence that agents such as catecholamines, acetylcholine, stretch (see below), Ca^{2+}, cyclic AMP, and cyclic GMP produce chronotropic and inotropic responses which are directionally con-

sistent. Could the sarcoplasmic reticulum be a developmental analog of the vesicles in pacemaking cells?

A second place where this scheme may be relevant is in subsidiary pacemakers of the heart. These include the atrioventricular node, which bears considerable structural similarity to the sinus node (*75*); possibly the catecholamine-containing "chromaffin" cells scattered throughout the right atrium (*68, 76*) for which no function has yet been identified; and perhaps also the well-studied Purkinje fibers, which under certain experimental conditions can also be induced to exhibit spontaneous activity. Although these latter cells contain large endogenous stores of catecholamines (*5*), pharmacological response differences between Purkinje fibers and natural pacemakers are legion (*1*), and may obscure any potential relevance of the mechanisms considered here.

Stretch and Synchronization

An important, but not widely recognized, modulator of pacemaker action is stretch. In mammalian sinus node, stretch increases the frequency of discharge (*77*), an effect which is mediated by an increase in the rate of spontaneous depolarization. This action can be sufficiently strong to induce spontaneous activity in those cells within the sinus node in which activity had been absent prior to stretch (*1*).

The ubiquity of the positive chronotropic effect of stretch is documented in Jensen's monograph on heart rate regulation (*78*). In hearts of the more primitive phyla, particularly mollusks and arthropods, the distension caused by the filling of the cardiac chambers is often the physiologic "trigger" of pacing; that is, unless sufficiently stretched some pacemakers will not pace.

Though the influence of stretch is evidently profound, a clear hypothesis to account for its action has not yet emerged (*1*). In view of the pre- and postsynaptic roles ascribed to pacemaking cells, it is tempting to look toward the synapse for analogous responses to stretch. At the myoneural junction, where the action of stretch is best documented, stretch increases the presynaptic vesicle discharge rate (*79*). The action is instantaneous (*80*), and the sensitivity to stretch is exquisite: stretch of 10 to 15 percent, depending upon the frequency of stimulation, increases the probability of vesicle discharge by five to ten times (*81*).

If an analogous situation existed in pacemaking cells, then the rate of spontaneous depolarization, according to the scheme described above, would be accelerated by stretch; in those cells in which the potential for spontaneous activity had not been realized by virtue of an insufficient discharge rate, stretch could provide the enhancement sufficient to initiate spontaneous activity.

Implicit in this action of stretch is a possible means by which one pacemaking cell within the sinus node might communicate with another. The many pacemaking cells which comprise the sinus node begin depolarizing spontaneously at rates which are quite independent of one another (*82*). Yet the sinus node acts as a single pacemaker: the ultimate action of the primary (fastest) pacemaking cell somehow causes the latent pacemaking cells to complete their cycle in an accelerated fashion, so the output signal from the sinus node is a synchronous one. The mechanism by which synchronization occurs is another of the unsolved mysteries of pacemaking (*1*).

It does not seem possible to solve this mystery by invoking electrical communication. Although most cells of the heart communicate electrically through low resistance intercellular gap junctional channels (*83*), these channels are rare or absent between cells of the sinus node (*13, 14*). Long-lasting potential gradients of substantial magnitude develop between neighboring pacemaker cells during the period of spontaneous depolarization (*83*), an observation difficult to reconcile with tight electrical coupling between cells. Nonetheless, if depolarizing current could flow from an excited to an unexcited sinus node cell, it probably would be of insufficient magnitude to elicit active depolarization. Ushiyama and Brooks (*84*) measured the amount of current required to elicit active depolarization by injecting depolarizing currents intracellularly. Although reasonable threshold values were obtained in various cardiac tissues, they could not excite sinus node cells, even when depolarizing currents were raised to levels sufficient to bring about cell destruction. Thus, even if some electrical coupling of sinus node cells existed, as implied by voltage clamp studies (*85*), it is difficult to imagine that such coupling could mediate synchronization.

Could synchronization occur mechanically? Contractile machinery is present in sinus node cells, though the myofilament packing density is considerably lower than in "working" myocardium (*13*). Contractile activity of the sinus node has been observed and measured (*86*). Since the primary pacemaking cells depolarize first, presumably they also contract first. Neighboring latent pacemaker cells, as yet unactivated, offer little resistance to stretch; stretch should abruptly accelerate their slow spontaneous rate of depolarization, and could thereby induce full activation. In such a way an excitation signal could propagate through the sinus node and synchronize the action of its constituent pacemaking cells.

One powerful feature of such a communication mechanism is its inherent safeness. As the number of depolarized cells increases, a larger proportion of the cells is stretching the remaining smaller proportion; the "gain" increases progressively. Once a critical fraction of the cells is contracting, stretch of the remaining cells should be sufficiently vigorous to ensure activation of these cells. Thus synchronization is achieved by an effectively regenerative mechanism. Yet the safeness inherent in the redundancy of multiple independent pacemaking cells is retained.

Although the mechanism of communication by stretch represents a secondary speculation, upon which the validity of the primary pacemaking mechanism does not depend, once invoked, certain basic features of sinus node function are readily accounted for:

1) The rising exponential waveform of depolarization, that is, the true pacemaker potential, is found only in primary pacemaking cells but not in latent pacemaking cells; in the latter, a slow spontaneous depolarization gives way abruptly to a sharply increased rate of depolarization (*1, 87*). These differences are predictable, as described above.

2) Propagation between primary and latent pacemaking cells of the sinus node occurs at a speed two orders of magnitude lower than in tissues where propagation is electrically mediated (*88*). Propagation by stretch is limited in speed by the time required to activate the myofilaments and by the maximum velocity with which the cells can contract, factors which are not relevant in electrically mediated propagation.

3) The speed and efficacy of intercellular communication are impaired by acetylcholine, vagal stimulation, Mn^{2+}, electrical overdrive, and hypothermia (*1, 87–89*). The common element among these agents is their tendency to diminish the rate of vesicle discharge, or the speed of contraction, or both. Consequently, such agents would be expected to impair synchronization. By extension, any agent which is both negatively chronotropic and negatively inotropic should impair synchronization.

4) Synchronization is improved by stretch (*1, 28*). This is in accord with the positive inotropic and positive chronotropic action of stretch.

5) Other communication-dependent

pacemaking phenomena such as pacemaker shift, decremental propagation, subthreshold oscillations, and local block [see (*1*)] can be reconciled with this mechanism, but detailed treatment is beyond the scope of this article.

While the stretch mechanism accounts for many features of intercellular communication, other mechanisms could act in a complementary manner. For example, within the proposed pacemaking scheme communication might also occur by chemical diffusion. Of the catecholamines discharged from primary pacemaking cells, particularly during the burst in step 3 (Fig. 2), some could diffuse to contiguous latent pacemaking cells and enhance depolarization. A local synchronizing action of this sort could complement the more global synchronizing action of stretch.

The stretch mechanism may have relevance in other tissues in which contraction occurs but electrical communication is weak or absent. The stretch mechanism has already been implicated in slow propagation through the atrioventricular node (*90*). Smooth muscle cells, like pacemaker cells, are difficult or impossible to excite by passage of intracellular depolarizing currents (*91*). On the other hand, stretch evokes a response that roughly parallels that occurring during the onset of spontaneous contraction: a fall in membrane potential and initiation, or increase in frequency, of the spike discharge (*92*). In those smooth muscle tissues relatively free of intercellular gap junctional channels (*93*), particularly those in which slow peristaltic contractions occur, stretch could play an additional role in mediating intercellular propagation. Such a possibility was suggested some time ago (*94*), but it seems to have escaped general attention.

Conclusion

It is apparent that a relatively simple hypothesis which accounts for the basic features of pacemaking can be constructed from several important, but not widely appreciated, clues. The mechanisms considered here offer avenues of approach to solutions of the two "mysteries" of pacemaking—the origin of spontaneous activity and the manner in which the spontaneous activities of individual pacemaking cells are coordinated to produce a synchronous output to the heart. Implicit in these mechanisms is a possible basis for understanding the genesis of some cardiac arrhythmias.

If experience is a guide, the specific mechanisms considered here may ultimately be proved invalid. However, alternative proposals which are broadly consistent with available data are lacking at present. Therefore, it seems reasonable to suggest that these mechanisms be considered as tentative working hypotheses, open to experimental confirmation or refutation.

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95. Many colleagues have given constructive criticism; I hesitate to list them for fear of inadvertently omitting one or two. However, special thanks are due to H. ter Keurs and B. R. Fink for their very helpful suggestions.

NEWS AND COMMENT

National Academy of Sciences: How the Elite Choose Their Peers

Philip Handler, president of the National Academy of Sciences, was drawing to the close of his annual report to the members on 26 April when he touched on the subject of election to the prestigious academy. Just that morning the members had completed their secret, mysterious, year-long election rites by voting in 60 new members "in recognition of their distinguished and continuing achievements in original research." Now Handler took note that there are pressures on the academy to broaden the pool of scientists from which it selects members. "The external world views us with a cocked eyebrow," he warned. "Egalitarianism and populism are in flood. There are those who would urge upon us public nomination of candidates for membership in the Academy. . . . There are . . . those who would have the membership of the Academy reflect the proportions of various groups within the population; by states, by sex, by ethnic groups. And there are those who consider the Academy to be an elitist relic of the past.

"Perhaps so," Handler continued. "But the hallmark of the Academy must continue to be excellence in all things and we must, above all else, retain our single criterion for election. We do believe that in science the best is vastly more important than the next best." A few sentences later Handler concluded. The members rose to give him a sustained standing ovation—the most heartfelt applause, some say, that Handler had ever received for an annual report. His defense of excellence and of the academy's ability to recognize it had touched a responsive chord.

But how well does the academy's labyrinthine elections process actually perform when it comes to selecting the most accomplished scientists in the land for membership? "That's a valid question," says David R. Goddard, the academy's home secretary and chief elections official. "I wish to hell I could give you an objective answer to it. We don't have an objective measure that I really know of." Goddard said it is his subjective opinion that "you can always find people outside the Academy who are just as good as the Academy members." But he described the number of "mediocre people" who gain admission to the academy as "minimal—there are relatively few Academy members that one would say shouldn't have been elected." However, with the academy membership now exceeding 1200, he added, even a 10 percent error rate "would leave you with a large number of ordinary people."

One imperfect measure of the academy's perspicacity is the extent to which it recognizes and honors talent of Nobel prize quality. (The measure is imperfect, among other reasons, because the Nobel judges are by no means infallible.) According to Goddard, "the Academy often has a red face" when the Nobel awards are announced and it turns out that some American scientist has been honored who is not yet a member of the academy. The 1976 Nobel prize in physics went to Burton Richter, of the Stanford Linear Accelerator Center, and Samuel C. C. Ting, of the Massachusetts Institute of Technology, neither of whom were members of the academy at the time. Both were elected to the academy at the recent meeting. The Richter-Ting case is understandable in that their Nobel prize came unusually soon after the work for which they were honored. But since 1950 there have been 12 other American scientists who received the Nobel prize but were not members of the academy at the time.

Many of these individuals appear to have been missed because they came from disciplines or institutions that were not strongly represented in the academy. Thus, in the past few years, the Nobel prize has been awarded to such non-academicians as Simon Kuznets and Wassily Leontief, both economists, a field in which the academy has only recently been building up its strength; Leon N Cooper, a physicist from Brown University, outside the mainstream of elite institutions which dominate the academy; and Ivar Giaever, an applied physicist from industry (General Electric), who was thus outside the community of pure academic scientists who predominate in the academy. In all of these cases, the academy subsequently elected the Nobelist to membership. Another industrial scientist who may have been overlooked unjustly for many years is Lewis H. Sarett, a chemist and president of Merck Sharp & Dohme Research Laboratories, who synthesized cortisone by various routes in 1944 and 1952 and won the National Medal of Science last year; he was finally elected to the academy this year. On an overall basis, however, Goddard believes that the academy does a respectable job in selecting the nation's most outstanding scientists. He notes that some 68 living academicians have won Nobel prizes and that "relatively few" of these got their Nobel award before their academy membership.

The chief criterion for election to the academy has traditionally been outstanding original research work. But there are no written guidelines defining just what a scientist must do to qualify, and other factors sometimes influence the academy electorate.

Some individuals appear to be elected because of their eminence as administra-