dose of the antagonist in moles per kilogram which will produce a doubling of the dose of the agonist required to produce the desired effect. The assumptions underlying the use of this procedure in vivo have been discussed (5). [See also A. E. Takemori, *Adv. Biochem. Psychopharmacol.* **8**, 335 (1974)].

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6. The pA_2 for antagonism by naloxone of the hyperthermic effects of morphine has been shown to be 6.6 and for respiratory depression 7.4 [K. L. McGilliard, F. C. Tulunay, A. E. Takemori, in *Opiates and Endogenous Peptides*, H. W. Kosterlitz, Ed. (North-Holland, Amsterdam, 1976), pp. 281–288]. For analgesia, pA_2 values ranging from 6.9 to 7.2 have been reported in rodents [V. Hollt, J. Dum, J. Blasig, P. Schubert, A. Herz, *Life Sci.* **16**, 1823 (1975); S. E. Smits and A. E. Takemori, *Br. J. Pharmacol.* **39**, 627 (1970)] and in primates (5).
7. Microelectrodes were glass micropipettes filled with 3*M* KCl, with impedances of 4 to 6 megohms.
8. The maximum degree of morphine depression of cell discharge was usually reached within 4 to 8 minutes, and the depression remained stable for approximately 20 to 30 minutes, depending on the dose. For this reason, a standard interval of 10 minutes between morphine doses was consistently employed. Naloxone, being more lipid soluble, has a more rapid onset and produced a maximum degree of antagonism after 3 to 4 minutes. Naloxone injections were therefore separated by 5 minutes. In previous experiments (2), naloxone at the highest dose used in these experiments (100 μg/kg) was observed to have none or only a minor effect on either the spontaneous activity or the magnitude of the Aδ and C fiber–evoked component. The effect was variable and not associated with any particular population of cells.
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Cardiac Pacemaking

The idea of Pollack (*1*) that sinus cells release catecholamines, thereby initiating and maintaining pacemaker activity, is not supported by experimental evidence. Two necessary steps in the sequence of events that keeps sinus cells firing are, according to Pollack: (i) interaction of catecholamines with sinoatrial β receptors and (ii) stimulation of the adenylyl cyclase. For (i) he quotes the experimental finding that a $10^{-5}M$ concentration of the β-receptor antagonist propranolol causes modest negative chronotropic effects in the neonatal mouse and arrest of cultured beating cardiocytes. From published experiments (*2*) it can be calculated that as little as $3 \times 10^{-9}M$ concentrations of racemic propranolol occupies 50 percent of sinoatrial β receptors stimulated by catecholamines. Similarly, $3 \times 10^{-9}M$ propranolol produces 50 percent occupancy of β receptors coupled to the adenylyl cyclase of cardiac membranes (*3*, *4*). However, $3 \times 10^{-9}M$ to $1 \times 10^{-6}M$ propranolol does not slow sinoatrial beating (*2*). Exposures of right atria to $10^{-6}M$ propranolol causes 99.7 percent β-receptor occupancy and requires a 300-fold increase of catecholamine concentration to achieve the same positive chronotropic effect as in the absence of propranolol. Thus, if the catecholamine release postulated by Pollack maintains beating rate, the sinoatrial cells in $10^{-6}M$ propranolol should release 300 times more catecholamine to keep their beating rate undiminished. This would imply an extraordinary adaptability of the pacemaker cell in furnishing the precise amount of catecholamine required to keep beating rate constant. It would also mean that such adaptability should be nearly instantaneous, because no fast transient decrease and reestablishment of beating frequency is observed with the administration of propranolol at a concentration of $10^{-6}M$. The depression or suppression of beating rate caused by the very high concentration ($10^{-5}M$) of propranolol quoted by Pollack is merely due to a toxic effect (*5*), unrelated to the drug–β-receptor interaction. Another high-affinity β-receptor antagonist, (−)-bupranolol (KL 255) (*3*) does not cause significant changes of beating frequency in cultured myoblasts (*6*) and right atria (*7*) from rats, while yielding 98.0 and 99.9 percent β-receptor occupancy, respectively. The commented evidence with (±)-propranolol and (−)-bupranolol strongly supports the view that sinoatrial and myoblastic cells can generate spontaneous beating activity when their β-receptor–catecholamine interaction is prevented. This is out of line with Pollack's suggestion of an obligatory role of sinoatrial catecholamines for eliciting automatism. Besides, an obligatory role of catecholamines for myocardial pacing would be catastrophic to the numerous patients who receive propranolol and other β-receptor antagonists as treatments for various cardiovascular diseases.

One should be cautious about accepting the contention of some authors quoted by Pollack that the beating rate of cultured cardiocytes under the influence of cardioactive drugs is directly proportional to the cellular concentration of adenosine 3′,5′-monophosphate (cyclic AMP). For instance, while submaximally effective concentrations of catechols cause an equilibrium increase of beating rate in myoblasts, their cellular content of cyclic AMP increases and quickly decreases again during steady state chronotropic effects (*7*). This evidence is inconsistent with a simple causal relationship between levels of cellular cyclic AMP and positive chronotropic effects of catecholamines.

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19 May 1977

Pollack (*1*) favors the view that catecholamines have an obligatory role in cardiac pacemaker activity. He bases this largely on the fact that isolated hearts or cultured heart cells cease to beat after treatment with reserpine. He does not review numerous articles which describe effects on heart rate of full blocking doses of β-adrenergic blocking agents, ganglionic blocking agents, or of atropine, given alone or in combination, to intact animals.

When the mammalian heart in situ, which is exposed to many substances not present in solutions used to support isolated tissues, is made unresponsive to injected catecholamines (and to acetylcholine) by blocking agents, the rate becomes quite constant. Cardiac arrest, or even bradycardia, does not occur. Pollack could say that although effects of exogenous catecholamines are blocked, endogenous catecholamine still is needed to maintain the heart beat. However, he theorizes that the endogenous catecholamine is passed out of the cell by exocytosis and then it exerts its action. It seems that a β-adrenergic blocking agent that would prevent the action of a substance arriving by the blood stream would also block the action of the

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same substance which is being released from the cell into the interstitial fluid in order to exert its action.

One reasonably may postulate that the effect of reserpine in causing arrest of pacemaker activity in cells in artificial solutions is related to lack of something other than catecholamines or that arrest is an effect of reserpine other than the catecholamine-depleting effect.

At present, the evidence that catecholamines are not necessary to sustain pacemaker activity seems much more convincing than the evidence for their having an obligatory role. Many of the actions of catecholamines described by Pollack have been well demonstrated, but it is not necessary to postulate that these actions are needed for maintenance of pacemaker activity.

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The central issue raised by Kaumann and Youmans is the following: If catecholamines play an obligatory role in pacemaking, as the bulk of the data have led me to conclude, why should therapeutic concentrations of β-adrenergic blocking agents not stop the heart, or at least substantially diminish its beating frequency?

Kaumann argues that therapeutic doses of propranolol ought effectively to saturate the sinoatrial β receptors, thereby cutting off essentially any interaction between catecholamines and the β receptors. However, he ignores the results of recent experiments with catecholamines covalently linked to glass beads [for example, (*1*)]. These experiments have shown that activation of a minute fraction of available β receptors can give maximal responses. Such propagated, or regenerative, responses are not amenable to treatment by classical receptor theory; consequently, Kaumann's computations of receptor occupancy do not carry much weight.

The question remains, however, why therapeutic doses of propranolol should have no negative chronotropic effect, if catecholamines, acting through β receptors, play an obligatory role in pacemaking. I regret not having discussed this in my article, since several colleagues have raised the same question. This "anomalous" effect may be readily explained on the basis of the distinctive microanatomy of the sinus node.

Unlike atrial cells, which are surrounded by a relatively large extracellular space, primary pacemaking cells appose one another very closely. Their surfaces undergo many infoldings, forming tortuous, intercellular clefts on the order of 100 Å wide (*2*, *3*). These cells are tightly packed into clusters, each of which is surrounded first by a basement membrane, and thence by a dense thicket of connective tissue (*2*, *3*). Thus the "extracellular space" between primary pacemaking cells—where I propose the endogenous catecholamines act—are narrow clefts which are relatively remote from the overall extracellular space. The situation is analogous to the one at the myoneural junction; although the cleft is "extracellular," the concentration of transmitter rises transiently far above the concentration in the extracellular space.

The limited accessibility of the space between primary pacemaking cells has obvious implication with regard to substances diffused in the extracellular space. Evidently such substances will reach cells whose surfaces are accessible to the extracellular space more readily than they will reach cells whose surfaces straddle narrow clefts. Within the sinus node there is a spectrum of cell types, ranging from the primary pacemaking cells, densely packed deep in the node, to atrial-like cells at the periphery; in between these are the transitional or latent pacemaking cells which are morphologically intermediate between atrial and primary pacemaking cells (*2*). Surface accessibility is therefore grossly divergent among the various cell types.

Now let us suppose catecholamines are infused into the sinus node. From the morphology described above, one would expect the catecholamine action to be more pronounced on latent, rather than primary, pacemaking cells. If this explanation is correct, catecholamine infusion should increase the rate of spontaneous depolarization preferentially in the latent pacemaking cells to the point where they take over as the dominant pacemaker, firing at a rate higher than control. Such "pacemaker shifts" are indeed found when the sinus node is exposed to exogenous catecholamines (*4*). Therapeutic doses of propranolol, therefore, merely block the chronotropic action of catecholamines on the latent pacemaking cells. Consequently, there is no reason to expect that propranolol, alone, should necessarily exert any negative chronotropic action on primary pacemakers.

In conclusion, the absence of negative chronotropic action of β-adrenergic blocking agents does not conflict with the hypothesis that catecholamines play an obligatory role in pacemaking.

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