tering isoproterenol (25 mg/kg, subcutaneously). Twenty-four hours later, the animals were anesthetized with sodium thiobutabarbital (Inactin, Buyk; 80 mg/kg, intraperitoneally) and an ultraminiature pressure transducer (model PR-249, Millar Instruments) was advanced into the left ventricle through the right carotid artery (*18*). Heart rate, left ventricular pressure, and maximum rate of increase in left ventricular pressure were continuously recorded on a multichannel Beckman RM Dynograph or a Gould Brush 2600 recorder. All functional parameters and the levels of ATP and adenine nucleotides were depressed (Table 1).

To determine whether ribose can stimulate cardiac adenine nucleotide biosynthesis in this pathophysiological condition, ribose or saline was infused in unanesthetized, unrestrained rats (*19*). When saline was infused, adenine nucleotide biosynthesis was enhanced, but only by about 100 percent (Table 2). But when ribose was administered, the increase in biosynthesis was comparable to that observed in other pathophysiological situations (*12–15*). As a result, ATP and total adenine nucleotides were essentially normal (Table 1). Likewise, the hemodynamic parameters were close to the control values, except for the product of left ventricular systolic pressure and heart rate (*20*), which was some 10 percent below control, since heart rate was still slightly reduced. Since ribose itself had no effect on cardiac hemodynamics (*18*), it is likely that the improvement in heart function resulted from the normalization of cardiac adenine nucleotides brought about by ribose. The exact site of ATP action remains unknown. It may be that ATP controls cardiac contractility primarily through its modulatory effects on calcium entry into the myocardial cell (*21*) or on calcium efflux from the sarcoplasmic reticulum (*22*).

The procedure used in this study is essentially a modification of a model in which cardiomyopathy is induced by catecholamines in open-chest rabbits (*23*). That catecholamine administration and constriction of the abdominal aorta significantly reduced all hemodynamic parameters in the closed-chest rat is not surprising, since catecholamine infusion alone impairs cardiac pump function (*23*). Of greater interest is the observation that the depression of heart function was prevented by ribose. Thus ribose qualifies as a cardioprotective agent. Its effect is primarily the stimulation of cardiac adenine nucleotide biosynthesis to such a degree that ATP and total adenine nucleotides are kept at normal levels.

Table 2. Rate of myocardial adenine nucleotide biosynthesis under control conditions and 24 hours after aortic constricton (AC) and subcutaneous injection of isoproterenol (ISO; 25 mg/kg) in rats intravenously infused with 0.9 percent NaCl or ribose (200 mg/kg per hour). Infusion rate: 5 ml/kg per hour. Measurements were done after exposing the rats to [1-^{14}C]glycine (250 μCi/kg, intravenously; specific activity, 54.2 mCi/mmole; Amersham) for 60 minutes in vivo (*25*). Values are means ± standard errors for the number of experiments given in parentheses.

Treatment	Adenine nucleotide biosynthesis (nmole/g per hour)
Control	6.6 ± 0.6 (31)
AC + ISO + NaCl	14.0 ± 1.3 (3)
AC + ISO + ribose	76.7 ± 0.3 (3)

These results support the hypothesis that ATP deficiency is primarily responsible for the impairment of cardiac contractility (*24*), since such impairment can be reversed when the decline in myocardial ATP is prevented. This approach may be an appropriate intervention in clinical states of depressed heart function due to energy deficiency. The advantage of ribose over other metabolic interventions is that it does not affect the hemodynamics of the heart with an ultimate change in oxygen demand and that it has no vasoactive properties which may result in afterload alterations.

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24 September 1982

Adenylate Cyclase Activation Shifts the Phase of a Circadian Pacemaker

Abstract. *Forskolin, a highly specific activator of adenylate cyclase, produced both delay and advance phase shifts of the circadian rhythm recorded from the isolated eye of the marine mollusk* Aplysia. *The phase dependence of the response to forskolin was identical to that with serotonin, which also stimulates adenylate cyclase in the eye. The ability of agents to activate adenylate cyclase in homogenates was correlated with their ability to shift the phase of the circadian oscillator. These results along with earlier findings show that adenosine 3′,5′-monophosphate mediates the effect of serotonin on the rhythm and regulates the phase of the circadian pacemaker in the eye of* Aplysia.

In the last decade significant progress has been made in understanding the physiology of circadian systems (*1, 2*). Although circadian pacemakers have been localized in the nervous systems of a number of multicellular organisms, the cellular and biochemical mechanisms that regulate circadian oscillators are still elusive. Serotonin, a putative neurotransmitter in the eye of *Aplysia* (*3*), causes a phase shift in the oscillator that is responsible for the circadian rhythm of spontaneous nerve impulses from the isolated eye of this marine mollusk (*4*). We report that both serotonin and forskolin activate adenylate cyclase in the eye, and that both shift the phase shift of the circadian rhythm in the same way. These experiments and our earlier work with serotonin show that adenosine 3′,5′-monophosphate (cyclic AMP) mediates the effect of serotonin on the

rhythm. Thus, the phase of the circadian oscillator in the eye is apparently regulated by cyclic AMP.

Activation of adenylate cyclase was determined from the amount of cyclic AMP formed in crude homogenates of eyes (*5, 6*). The increase in the activity of adenylate cyclase elicited by serotonin was dependent on the dose (Fig. 1A). The effect of serotonin concentration on adenylate cyclase activation and on the formation of cyclic AMP in intact eyes (*7*) is similar. In both cases the threshold concentration was approximately $10^{-7}M$ serotonin. These results are consistent with the threshold at which serotonin causes a phase shift in the rhythm, which occurs between concentrations of $10^{-8}M$ and $10^{-7}M$ serotonin (*3*). Other amine neurotransmitters (dopamine, octopamine, and histamine), which are found in the eye (*3, 4, 8*) or nervous system of *Aplysia* (*9*), had no effect on adenylate cyclase activity (*6*) and also had no effect on the rhythm (*3, 10*).

The action of serotonin on adenylate cyclase in homogenates suggests that serotonin shifts the phase of the rhythm by activation of adenylate cyclase. To test this possibility, we examined whether forskolin, a highly specific activator of adenylate cyclase (*11*), mimicked the effects of serotonin on the enzyme and on the rhythm. The adenylate cyclase activity stimulated by forskolin was dependent on dose, with detectable increases in enzyme activity occurring at $10^{-6}M$ (Fig. 1B). Like serotonin, this agent did not affect phosphodiesterase or guanylate cyclase activity in the eye (*12*). These results are consistent with those found in other systems in which the specificity of action of forskolin is well documented (*11*).

To examine the effect of forskolin on the rhythm, isolated eyes were treated for 6 hours with $10^{-6}M$ forskolin at different phases of the rhythm. Phase shifts in rhythms were measured by comparisons of the peaks of the rhythms of matched experimental and control eyes during the third cycle after the treatment. Forskolin produced delay phase shifts (-3.1 ± 0.8 hours, $N = 4$, mean ±95 percent confidence interval) when eyes were treated during the early subjective day and advance phase shifts ($+2.0 \pm 1.1$ hours, $N = 4$) when eyes were treated during the late subjective day (Fig. 2, A and B). A curve relating the phase shifts produced by forskolin as a function of the phase of treatment is shown in Fig. 2C and, for comparison a phase response curve obtained by treating eyes at different phases with serotonin (*13*) is shown. The curves are indistinguishable, and their similarity is significant because most other treatments on the eye produce response curves that are very different from the one produced by serotonin (*13*).

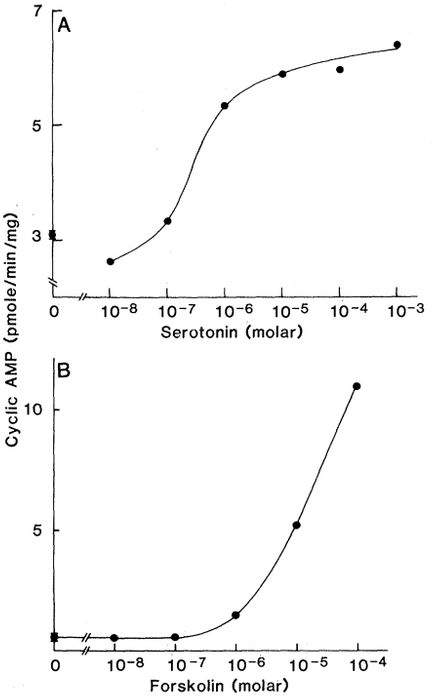

Fig. 1. (A) Serotonin-stimulated adenylate cyclase activity in homogenates of *Aplysia* eye. (B) Activation of adenylate cyclase activity by forskolin in *Aplysia* eye homogenates. Each point is the average of duplicate assays, and all assays contained $10^{-5}M$ GTP.

We have shown that 8-benzylthio–cyclic AMP mimics the effect of serotonin on the rhythm, that the effects of serotonin and 8-benzylthio–cyclic AMP are not additive, and that phosphodiesterase inhibitors potentiate the effect of serotonin (*7*). In intact cells of the eye, serotonin increases the concentrations of cyclic AMP (*7*). In broken cell preparations of the eye, low concentrations of serotonin and forskolin stimulate adenylate cyclase activity. Forskolin precisely mimics the effects of serotonin on the rhythm. These results satisfy the criteria outlined by Sutherland to show that a physiological effect is mediated by cyclic AMP (*14*).

We conclude that serotonin shifts the phase of the rhythm by stimulating adenylate cyclase and increasing cyclic AMP. Thus, the system that regulates cyclic AMP forms a portion of the pathway by which serotonin perturbs the circadian oscillator and shifts the phase of the rhythm. Cyclic AMP is the first intracellular compound to be identified that regulates the phase of a circadian oscillator (*15*).

Ultimately the entrainment pathway for serotonin must end at the circadian oscillator. Whether changes in cyclic AMP represent changes along the entrainment pathway, or whether the system regulating cyclic AMP concentrations in the cell forms a part of the circadian oscillating mechanism is not yet known. Elucidating the role of cyclic

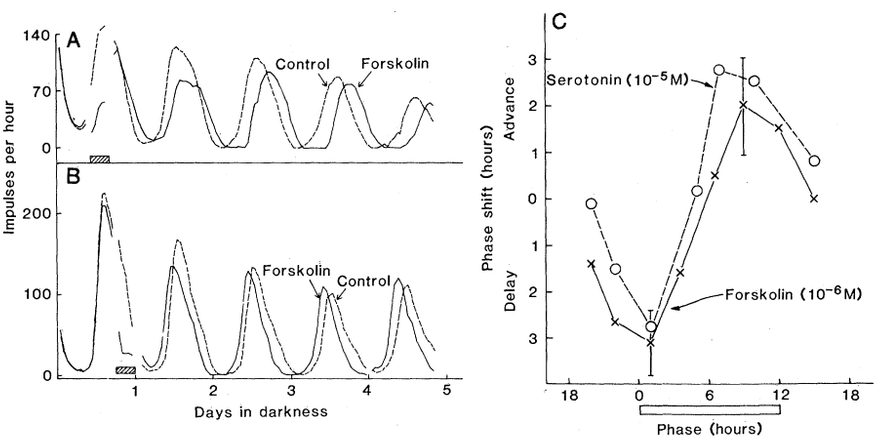

Fig. 2. Effects of forskolin on the phase of the circadian rhythm of the *Aplysia* eye. (A) Delay phase shift produced by 6-hour treatment with forskolin ($10^{-6}M$) during the time shown by the hatched bar on the bottom of the graph. The frequency of spontaneous optic nerve impulses is plotted as a function of the time isolated eyes were in constant darkness. Rhythms shown are from eyes of the same animal. The abscissa begins at projected dusk (at hour 12 circadian time) of the LD 12:12 cycle used to entrain the intact animals. (B) Advance phase shift produced by forskolin ($10^{-6}M$) given during a later time than the treatment in (A). (C) Comparison of 6-hour treatments of forskolin and serotonin. The graph shows phase shifts produced in the rhythm as a function of the time (phase) of exposure of the eyes to the treatments. Treatments were given during the first day of isolation of the eyes and phase 0 is the time of projected dawn. Data are plotted with respect to the midpoint time of the treatment. In the forskolin experiment, each point is the mean phase shift for three or four pairs of eyes. A total of 28 pairs of eyes were used in this experiment. The 95 percent confidence intervals are shown for the maximum delay and advance ($N = 4$). Data for serotonin are from (*13*).

AMP in the circadian pacemaking system at the biochemical level should aid in the search for the molecular components of the oscillator.

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Bidirectional Transmission at the Rectifying Electrotonic Synapse: A Voltage-Dependent Process

Abstract. *Rectifying properties of electrotonic synapses established by the crayfish giant motor fiber are associated with a more negative resting membrane potential in the presynaptic than in the postsynaptic side of the junction. An increased junctional conductance and bidirectional transmission are produced, with almost no delay, by inverting this polarization.*

Although electrotonic transmission through gap junctions is generally bidirectional (*1, 2*), in a few systems, the junctional resistance between coupled cells varies in such a way that currents flow more readily in one direction. In the crayfish giant motor synapse (GMS), spikes and passive depolarizations pass from the presynaptic lateral and medial giant axons to the postsynaptic giant motor fiber, whereas only hyperpolarizations spread in the opposite direction (*3, 4*). This property can be functionally significant (*4, 5*), but its origin remains

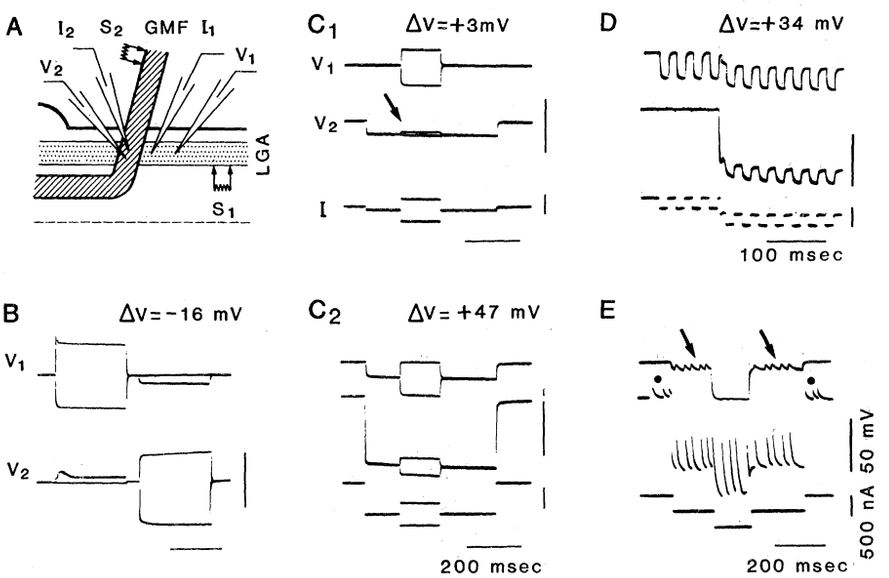

Fig. 1. Tests for the transmission of electrotonic potentials. (A) Diagram illustrating the microelectrodes (one for current passing, *I*, and one for voltage recording, *V*) inserted in the lateral giant axon (*LGA*, I_1, and V_1) and the giant motor fiber (*GMF*, I_2, and V_2) and the extracellular electrodes (S_1 and S_2). (B) Rectified transmission in resting conditions. (C to E) Voltage dependence of the rectification. Upper and middle traces: voltage recorded from presynaptic (V_1) and postsynaptic (V_2) cells; lower trace: superimposed current steps applied to pre- and postsynaptic sides of the junction. (C_1) During slight hyperpolarizations of the motor fiber, only positive pulses spread from the pre- to the postsynaptic side (arrow), with a calculated coupling coefficient *k* of about 0.16 (C_2). With stronger hyperpolarizations, synaptic transmission became bidirectional, *k* reaching 0.48 and 0.52 for positive and negative pulses, respectively. (D) Short negative pulses (25 msec) were applied to the presynaptic axon, and transynaptic spread of current began almost instantaneously with the onset of postsynaptic hyperpolarization. (E) Postsynaptic potentials occurring in the motor fiber (dots, middle traces) spread in the presynaptic axon (upper trace, arrows) during hyperpolarizations of the postsynaptic fiber moving ΔV from −17 to +53 mV. (A reduction of this potential difference by a short polarization of the lateral axon reversibly blocked the transynaptic effect, despite a concomitant increase in the amplitude of the postsynaptic potential.)