Circadian Rhythm of Serotonin in the Pineal Body of Immunosympathectomized Immature Rats

Abstract. *In the pineal body of the immature rat the circadian rhythm of serotonin persists when sympathetic innervation is abolished by the administration of nerve growth factor antiserum. This rhythm is regulated by a mechanism that does not involve the sympathetic innervation and is, therefore, fundamentally different from that in the adult.*

In the past 10 years major advances in our knowledge of pineal physiology have been established. Compounds within the pineal body have been identified and their levels measured. Many of them have been shown to have a circadian rhythm that is dependent upon environmental lighting mediated through the retina and the sympathetic nervous system (*1*).

Serotonin is one of these compounds. In the rat pineal it exists in high titers (*2*) and has a circadian rhythm in which levels are lowest 4 hours after the onset of darkness and highest 6 to 8 hours after the onset of light (*3*). Sympathetic postganglionic fibers from the superior cervical ganglion (*4*) regulate the rhythm. After bilateral superior cervical ganglionectomy or severance of preganglionic fibers the cycling of serotonin in the pineal body is abolished (*5, 6*). Although this role of the sympathetics has been confirmed in the adult rat, the following observations suggest that pineal serotonin may not be regulated through sympathetic innervation in the immature animal.

As early as 6 days postpartum the circadian rhythm of serotonin is present (*7*), but at this age there is a sparsity of intrapineal sympathetic nerve fibers (*8*). To learn the significance of this apparently inadequate innervation we undertook the study, described in this report, of serotonin levels in totally denervated pineals in young rats. Because of stress and mortality after superior cervical ganglionectomy, we chose to denervate the pineal by immunosympathectomy (*9*).

Holtzman rats of both sexes were given bovine nerve growth factor antiserum (NGFA) (*10*) within 6 hours after birth and again 24 hours later. Experimental and control animals were maintained together in a controlled environment. The temperature was 19° ± 1°C, and fluorescent lights were kept on from 5:00 a.m. to 7:00 p.m., that is, a cycle of 14 hours of light and 10 hours of darkness.

Animals were decapitated at either 8 or 20 days of age—one group at 1:00 p.m. and another at 11:00 p.m. Pineals were dissected quickly, weighed on saline-moistened filter paper on a Roller Smith torsion balance, homogenized in groups of two in 0.5 ml of 0.1*N* HCl and 0.5 percent ascorbic acid, and refrigerated. Within 24 hours serotonin was assayed on a Farrand spectrofluorometer (model No. 104244B) according to the method of Quay (*11*). Since fluorescence microscopy reveals the extent of sympathetic nerve suppression (*12*), a sample of pineal bodies from each group of treated animals was examined by this method. In addition, stretch preparations of the iris of all the 20-day-old animals used in the experiment were examined by fluorescence microscopy. In normal control pineals (from animals 8 and 20 days old), the sympathetic innervation was present and similar to that described elsewhere (*8*).

In animals injected with NGFA (600 unit/g) there was no nerve fluorescence in the iris preparations, and although denervation of the pineal was almost complete, occasionally a few scattered fibers remained. When the dosage was increased to 1100 unit/g, pineal nerve fluorescence was abolished.

Prolonging the light period into the normal dark period prevents the nocturnal fall in pineal serotonin in both adult and immature animals (*6, 7*). To clarify the role of sympathetics in the immature rat, it was important to determine whether prolonged lighting would modify the amount of serotonin in denervated pineals. Thus on the day the animals were killed a third group was given four additional hours of lighting, that is lights remained on until 11:00 p.m., the time they were killed.

Results in Table 1 show that at both 8 and 20 days of age there is a circadian rhythm in rat pineal serotonin and that after immunosympathectomy the rhythm persists unchanged. Thus its regulation must be through a mechanism fundamentally different from that in the adult.

In the adult, central nervous system stimuli that regulate serotonin levels reach the pineal via sympathetic fibers (*5*). Since rhythm persists in the young animal after denervation, it seems reasonable to speculate that in the immature rat the rhythm is intrinsic to the pineal itself. However, the origin and nature of its regulator remains unknown. An endocrine influence in immature rats has not been studied, but in the adult, removal of endocrine organs does not modify pineal serotonin rhythm (*6*).

The results of Table 1 also show that in sympathectomized animals additional lighting did not elevate serotonin levels above the nocturnal low. From the first observation—that serotonin rhythm persists in the absence of sympathetics—one might presume that innervation has no regulatory function on serotonin metabolism in the young rat. However, since additional lighting prevents the nocturnal fall in serotonin in intact

Table 1. Serotonin levels in the pineals of normal and immunosympathectomized young rats. Each group contained 10 or 12 animals. There is no significant difference between pineal weights of groups in each of the four treatment categories. N.S., not significant.

Treatment	Pineal weight (mg)	Serotonin (ng/pineal)	Significance of difference	
			Groups	Level
		Rats 8 days old		
None				
Day*	0.470 ± 0.035	30.5 ± 2.20	Day:night	.001
Night†	.502 ± .022	13.3 ± 1.60	Day:night-lighted	.02
Night-lighted	.410 ± .013	23.1 ± 1.50	Night:night-lighted	.01
NGFA (1100 unit g^{-1} day^{-1})				
Day	.473 ± .015	29.6 ± 1.63	Day:night	.001
Night	.527 ± .030	10.0 ± 2.33	Day:night-lighted	.001
Night-lighted	.483 ± .017	14.5 ± 2.78	Night:night-lighted	N.S.
		Rats 20 days old		
None				
Day	0.586 ± 0.039	54.5 ± 4.59	Day:night	.001
Night	.632 ± .037	13.5 ± 4.12		
NGFA (600 unit g^{-1} day^{-1})				
Day	.549 ± .031	61.0 ± 3.52	Day:night	.001
Night	.550 ± .034	19.0 ± 1.81		

* Day, 1:00 p.m. † Night, 11 p.m.

newborn rats, but immunosympathectomy blocks that response (Table 1), innervation seems significant in transmitting this effect of the additional lighting. Zweig *et al.* (*7*) reported that when lighting was prolonged 4 hours into the normally dark period serotonin levels equalled the daytime high. Our data show that such additional lighting produces an intermediate level that is significantly higher than the nocturnal low ($P < .01$) but significantly lower than the daytime peak ($P < .02$). This observation supports the contention that there may be both a nerve-dependent and a nerve-independent serotonin rhythm at this age in the rat (*13*).

In contrast to the adult, light stimulus in the immature rat can reach the pineal by a route involving the retina (*7*). The receptor for this route is located somewhere in the head, but a more specific localization is unknown (*7*). Since denervated glands do not respond to additional illumination, the receptor for the involved neural pathway is probably not within the pineal body itself, but within the central nervous system—perhaps the hypothalamus.

This report of a serotonin rhythm after immunosympathectomy has been confirmed in pineals from 20-day-old rats sympathectomized by superior cervical ganglionectomy at the age of 8 days (*13*).

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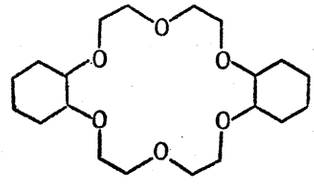
Binding of Alkali Metal Ions by Cyclic Polyethers: Significance in Ion Transport Processes

Abstract. *Values for the formation constant (log K), the change in enthalpy ($\Delta H°$), and the change in entropy ($\Delta S°$) have been determined for the interaction of lithium, sodium, potassium, rubidium, and cesium ions with the two isomers of the cyclic polyether, 2,5,8,15,18,21-hexaoxatricyclo[20.4.0.0^{9,14}] hexacosane. The stability order of these metal ions with either isomer is identical to the permeability order for these same metal ions with the structurally related antibiotics, valinomycin and monactin.*

Considerable research effort, particularly among biochemists and physiologists, has been directed toward understanding the phenomenon of ion transport through cellular membranes. One hypothesis suggests that carrier molecules are important in the active transport of ions. Experimental justification for this hypothesis is found in the fact that many substances markedly affect active ion transport in systems consisting of mitochondrial, chloroplast, or reconstituted biological membranes. Of special interest among substances affecting ion transport are cyclic molecules of low molecular weight containing residues joined by amide, ester, or ether linkages, or any combination of these linkages; for example, antibiotics of the valinomycin and actin classes and certain of the recently synthesized cyclic polyethers, such as 2,5,8,15,18,21-hexaoxatricyclo[20.4.0.0^{9,14}] hexacosane (*1*)



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We propose that compound I may serve as a model compound for thermodynamic investigations of active transport processes inasmuch as this compound (i) increases the permeability to alkali metal ions (*2*) of reconstituted biological membranes in the sequence

$$K^+ > Rb^+ > Cs^+ > Na^+ > Li^+ \qquad (1)$$

and (ii) is structurally similar to certain of the antibiotic molecules, such as valinomycin (*3*) and monactin (*2*), which also exhibit this property with the same metal ion sequence. Consequently, calorimetric titration and potentiometric studies of the interaction of alkali metal ions with the *cis* and *trans* isomers of compound I, IA and IB, have been initiated. Results show that sequence 1 also holds for the thermodynamic stabilities of the complexes formed by these metal ions with isomers IA and IB.

The sample of compound I (*4*) containing the isomer mixture was purified by passage through an acid-washed aluminum oxide column with heptane used as eluent. The isomers were then separated by passing the purified sample through a Woelm aluminum oxide column (activity grade 1) and varying the eluent among hexane-ether mixtures. The column was stripped with methanol, and the isomers were recrystallized from ether and hexane. Isomers IA and IB have melting points of 61° to 62°C and 69° to 70°C, respectively; they can also be distinguished by their infrared spectra, although it is not presently known which isomer is *cis* and which is *trans*.

Solutions of alkali metal salts (~0.2 and ~0.4*F*) were titrated into solutions of either isomer IA or IB (~0.01*F*) in a precision thermometric titration calorimeter (*5*). Values for the formation constant *K*, the change in enthalpy $\Delta H°$, and the change in entropy $\Delta S°$ were calculated from the calorimetric titration data (*6*). The calculations were aided by an IBM-360 computer.

Table 1. Thermodynamic quantities for the reaction $M^+ + I = MI^+$. Uncertainties are the standard deviations from the average of three to seven runs in each case.

Metal ion M^+	Isomer of compound I	log *K*	$\Delta H°$ (kcal/mole)	$\Delta S°$ (cal/deg mole)
K^+	A	2.01 ± 0.02	− 3.89 ± 0.06	− 3.8 ± 0.2
K^+	B	1.60 ± .03	− 5.18 ± .08	− 10.1 ± .3
Rb^+	A	1.47 ± .02	− 3.48 ± .06	− 5.0 ± .2
Cs^+	A	1.07 ± .06	− 2.00 ± .10	− 1.8 ± .3