surgical procedure. Such a relationship could help to account for the protective effect of regular physical activity against coronary artery disease in man (*14*) and in experimental animals (*15*), in that such conditioning results in lowered average heart rates (*16*) and therefore in a reduction in total number of heart beats for extended time periods. Lowered heart rate results in more total time spent in diastole when changes in the rate of flow and departures from laminar unidirectional flow are least. Conversely, the association of type A personality traits with coronary heart disease (*17*), and the experimental finding (*18*) that psychosocial stress in monkeys predisposes to coronary plaque formation, could both be the result of frequent stress-related elevations of heart rate that would tend to elevate average heart rate for extended periods. Although the mechanisms are not entirely understood, myocardial oxygen extraction is improved with regular physical activity or exercise training, and physically fit individuals have a lower baseline heart rate, a smaller increase in heart rate when stressed, and a more expeditious return to baseline than do untrained individuals (*19*). The resulting long-term reduced heart rate in physically trained individuals may also retard the progression of atherosclerotic lesions in their coronary arteries. The role of heart rate in the severity and distribution of atherosclerotic lesions merits further investigation.

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Rhodopsin Kinase Activity in the Mammalian Pineal Gland and Other Tissues

Abstract. *Rhodopsin kinase, an enzyme involved in photochemical transduction in the retina, has been found in the mammalian pineal gland in amounts equal to those in the retina; other tissues had 7 percent of this amount, or less. This finding suggests that, in mammals, rhodopsin kinase functions in the pineal gland and other tissues to phosphorylate rhodopsin-like integral membrane receptors and is thereby involved in signal transduction.*

Rhodopsin kinase (RK) is a soluble enzyme that selectively phosphorylates photon-activated rhodopsin (*1–3*). It is thought to play a critical adaptive role in photochemical transduction in the retina by reducing the potency of photon-activated rhodopsin to stimulate guanosine 3,′5′-monophosphate (cyclic GMP) phosphodiesterase (*4*). Rhodopsin kinase is generally considered a retinal enzyme, not present in other tissues. We now report that the mammalian pineal gland has RK activity equivalent to that observed in the retina; its activity in other tissues is less than 7 percent of that in the retina.

Rhodopsin kinase activity was measured by incubating crude homogenates or supernatants (30,000*g*) of rat tissues with [γ-^{32}P] adenosine triphosphate (ATP) and urea-treated rod outer segments (U-ROS) from bovine retina (*5*); phosphorylated opsin was extracted from the U-ROS and isolated by polyacrylamide gel electrophoresis in monomeric and dimeric forms (*6*) (Fig. 1). For quantitation, the monomer and dimer bands of opsin were identified and removed from the dried gel; the amount of radioactive label was then measured by conventional methods, and RK activity was calculated from the sum of the

Table 1. Rhodopsin kinase activity in selected rat tissues. Each assay was performed with about 5 μg of supernatant protein, [^{32}P]ATP (200 μ*M*), and U-ROS containing 0.5 nmol rhodopsin in 50 μl (*4, 5, 7*). The incubation period was 5 minutes.

Tissue	Activity (pmol·mg^{-1}·min^{-1})	Relative activity (percent of retinal activity)
Retina	193 ± 55	100
Pineal	131 ± 49	68
Cerebellum	13.7 ± 2.48	7.1
Cerebral cortex	13.1 ± 2.52	6.8
Hypothalmus	12.0 ± 1.72	6.2
Lung	9.0 ± 1.49	4.7
Olfactory lobe	7.7 ± 0.83	4.0
Brainstem	6.9 ± 0.76	3.6
Anterior pituitary	5.5 ± 1.11	2.8
Posterior pituitary	5.4 ± 1.31	2.8
Spleen	4.4 ± 0.80	2.3
Ovary	2.4 ± 0.29	1.2
Adrenal	2.3 ± 0.44	1.2
Liver	1.4 ± 0.78	0.6
Testes	1.1 ± 0.48	0.6
Harderian gland	0.67 ± 0.054	0.3
Kidney	0.45 ± 0.31	0.2

amount of label in both bands. The assay was linear over a range of 1 to 5 μg of crude pineal or retinal protein (*7*). In addition, an assay containing 5 μg of protein from either source was linear for up to 10 minutes, and activity was reduced if the assay was done under red light (Fig. 2A) to prevent photon activation of rhodopsin. We also found that pineal RK activity was inhibited by zinc (Fig. 2B), as is true of retinal RK (*2*). These characteristics provide evidence that pineal and retinal RK are similar, if not identical.

A quantitative tissue distribution study (Table 1) revealed that the pineal gland and retina contain similar amounts of RK activity and that the activity in other tissues, including the anterior and posterior pituitary glands, five brain regions, and lung is 4 to 7 percent of the retinal activity (*8*). Small amounts of activity were detected in the spleen, adrenal, and ovaries; barely detectable levels of activity were found in the testes, Harderian gland, and liver. Although it appears that pineal and retinal RK are the same molecules, it is possible that apparent RK activity in tissues other than the retina and the pineal gland represents the action of kinases with broader specificities.

The finding of high RK activity in the pineal gland is consistent with data indicating first, that the pineal gland contains a highly antigenic protein first discovered in the retina, termed the "S" antigen (*9*), and second, that retinal S antigen probably is the 52K form of RK (RK exists in 52K and 68K forms) (*2, 10*). This pineal-retinal similarity suggests to us that pineal RK may be an evolutionary holdover from the ancestral function of the pineal gland as a photoreceptor organ (*11*). Although the presence of an immunoreactive rhodopsin-like protein and direct photic stimulation of the pineal organ have been observed in the pineal glands of various teleost fishes, frogs, and some birds and turtles, the mammalian pineal gland does not contain rhodopsin-like immunoreactivity and is not known to be photosensitive (*11, 12*). However, the mammalian pineal gland is indirectly regulated by light via a retinal → pineal neural circuit, and it is possible that RK still functions in the photoregulation of this tissue.

The neural circuit controlling the mammalian pineal gland extends from the retina via a retinohypothalamic projection to the hypothalamic suprachiasmatic nuclei (SCN), a circadian clock system that controls circadian rhythms in a number of tissues, including the pineal gland. Light entrains the clock to the environmental lighting cycle and gates signals to the pineal gland (*13*). The SCN → pineal circuit passes through central and peripheral structures; the transmitter involved in the pineal gland is norepinephrine (*13–15*). At night in the dark, norepinephrine is released and acts through postsynaptic α_1- and β_1-adrenoceptors to stimulate pineal cyclic AMP (adenosine 3′,5′-monophosphate) and cyclic GMP (*15, 16*). Pineal β_1-adrenoceptors regulate adenylate cyclase activity; it is not clear how pineal α_1-adrenoceptors act on cyclic nucleotides. The increase in cyclic AMP has several metabolic consequences, including an increase in melatonin production.

A clue to the function of pineal RK may lie in the function of retinal RK and the knowledge that light is associated with a decrease in cyclic nucleotides in both tissues. From the evidence that retinal RK is involved in photochemical transduction and phosphorylates an integral membrane receptor for photons—that is, rhodopsin—we suspect that pineal RK is involved in neurochemical transduction and phosphorylates an integral membrane receptor for norepinephrine, an adrenoceptor. Similarly, be-

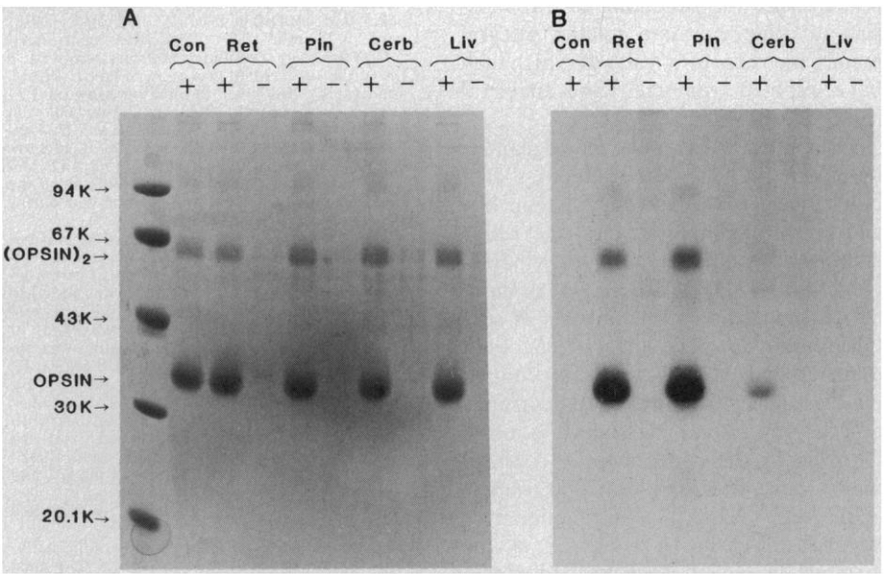

Fig. 1. Polyacrylamide gel analysis of 10,000*g* pellets from RK assays. (+) with U-ROS; (−) without U-ROS. Molecular weight standards are identified on the left; the locations of the opsin monomer and dimer are indicated. (A) Photograph of a Coomassie blue–stained gel; (B) autoradiograph (Osray RP1 Agfa-Gevaert) of dried gel. The exposure period was 48 hours; an intensifying screen (Kodak) was used. Abbreviations: Con, control; Ret, retina; Pin, pineal gland; Cerb, cerebellum; and Liv, liver.

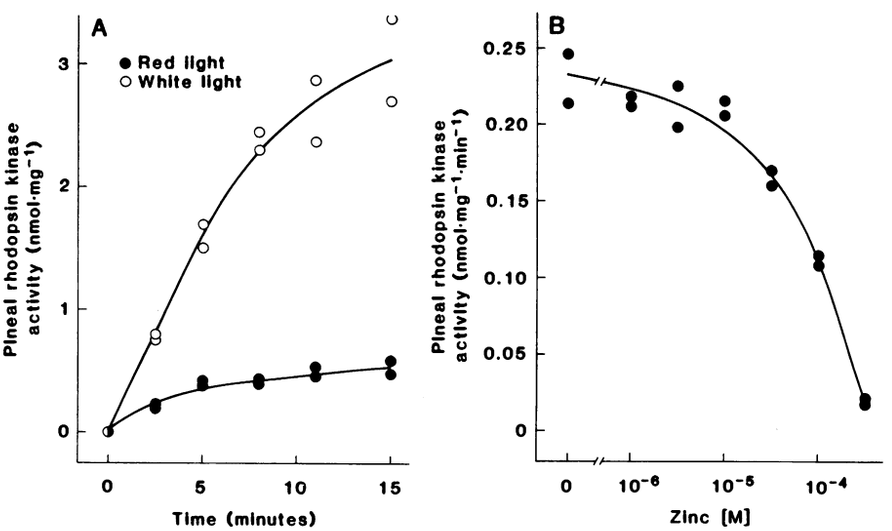

Fig. 2. Pineal RK activity. Samples of pineal homogenates (approximately 5 μg of protein) from 300-g rats were incubated with U-ROS (*4*). (A) Light dependence of rhodopsin phosphorylation by pineal RK. Tubes were incubated in a dark room with a dim red light or under white light in normal laboratory conditions. (B) Effect of zinc. Zinc chloride was added at the concentration indicated, and the mixture was incubated for 5 minutes.

cause retinal RK decreases the cyclic nucleotide response to photon stimulation (*4*), we suspect that pineal RK is involved in the regulation of cyclic nucleotide responsiveness of pinealocytes to adrenergic stimulation (*15, 16*). Finally, because rhodopsin regulates phosphodiesterase activity, we speculate that the pineal adrenoceptor substrate of RK regulates phosphodiesterase. The phosphorylation of β-adrenoceptors associated with modulation of responsiveness has been reported (*17*); the kinase involved might be RK.

The presence of RK activity in diverse tissues suggests that this, or a closely related, enzyme functions in these tissues to phosphorylate related integral membrane receptors, each of which may have evolved from the same ancestral receptor molecule.

The finding of RK in the pineal gland is only one in a series of findings that biochemically link the mammalian eye and pineal gland. First, both the retina and pineal gland contain hydroxyindole-*O*-methyltransferase, an enzyme of limited distribution, which converts *N*-acetylserotonin to melatonin (*18*). Second, as mentioned above, both tissues contain the S antigen (*9*). Third, the occurrence of bilateral retinal blastomas is often followed by the appearance of a pineal tumor—a syndrome described as trilateral blastoma (*19*). Although function may have changed during evolution as the mammalian pineal gland lost its photoreceptor function and became entirely neurosecretory (*11*), both the retina and pineal gland are biochemically more similar in mammals than is generally acknowledged. Recognition of this relation might allow pineal and retinal biochemists to benefit from advances in each other's field.

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5. Tissues obtained from 250-g male rats (Sprague-Dawley) were prepared by homogenization (1 mg of wet weight per 20 μl) in 10 m*M* Pipes, containing 4 m*M* dithiothreitol and 1 m*M* EDTA (*p*H 7.0). The kinase assay contained, unless otherwise indicated, 50 m*M* potassium phosphate (*p*H 6.8), containing 1 m*M* $MgCl_2$, 0.1 m*M* [^{32}P]ATP (1×10^6 to 2×10^6 cpm/nmol), 0.5 nmol of rhodopsin in urea-treated bovine rod outer segments [H. Shichi, R. L. Somers, K. Yamamoto, *Methods Enzymol.* **99**, 362 (1983)], and 5 μg of supernatant protein. The final volume of the assay was 50 μl. The assay was stopped by the addition of 200 μl of 0.125*M* potassium phosphate buffer (*p*H 6.8), containing 62.5 m*M* potassium fluoride, 12.5 m*M* EDTA, and 6.25 m*M* ATP. This was centrifuged (10,000*g*), and the supernatant was removed; 2 μl of the supernatant was analyzed for [^{32}P]ATP content by thin-layer chromatography on PEI cellulose (E. Merck), with 1*M* lithium chloride used as the solvent [K. Randerath and E. Randerath, *J. Chromatogr.* **16**, 111 (1964)]. Identification and measurement of the [^{32}P]ATP and $^{32}PO_4$ indicated that less than 20 percent of the ATP originally present was hydrolyzed during the assay.
6. The pellet was dissolved in 40 μl of 10 m*M* tris acetate (*p*H 7.4), containing 2.5 percent sodium dodecyl sulfate (SDS), 2 percent β-mercaptoethanol, and 5 m*M* EDTA. To this was added 10 μl of 50 percent glycerol with 0.05 percent bromophenol blue. The sample was kept at room temperature, and a 25-μl sample was applied to a 10 percent acrylamide-SDS gel [U. K. Laemmli, *Nature (London)* **227**, 680 (1970)]. Electrophoresis was for 30 to 40 minutes at 60 V and then for 3.5 hours at 165 V (constant voltage). The gel was stained, dried, and the [^{32}P]phosphorylated opsin bands were visualized by autoradiography, cut out of the gel, and counted.
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8. To determine the tissue distribution of RK activity, we removed tissues from 250- to 300-g male rats; ovaries were removed from 250-g female rats. The tissues were immediately placed on solid CO_2 and stored (−70°C). Tissues were thawed individually, and small pieces were weighed and homogenized (20 μl per milligram of wet weight) in 10 m*M* Pipes (*p*H 7.0), containing 4 m*M* dithiothreitol, 1 m*M* EDTA, leupeptin (20 μg/ml), and 0.3 m*M* phenylmethylsulfonyl fluoride. The samples were centrifuged (30,000*g*) for 30 minutes, and the supernatant was removed and stored at −70°C. The protein content was measured, and a sample of the supernatant containing 5 μg of protein was assayed as described (*4, 5*), with 0.2 m*M* [^{32}P]ATP and 10 μ*M* rhodopsin.
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Caffeine and Related Methylxanthines: Possible Naturally Occurring Pesticides

Abstract. *Natural and synthetic methylxanthines inhibit insect feeding and are pesticidal at concentrations known to occur in plants. These effects are due primarily to inhibition of phosphodiesterase activity and to an increase in intracellular cyclic adenosine monophosphate. At lower concentrations, methylxanthines are potent synergists of other pesticides known to activate adenylate cyclase in insects. These data suggest that methylxanthines may function as natural insecticides and that phosphodiesterase inhibitors, alone or in combination with other compounds, may be useful in insect control.*

The methylxanthines, including caffeine and theophylline, are found in the berries, seeds, and leaves of a number of species, including tea, coffee, cocoa, and kola (*1*). Although methylxanthines are frequently used as stimulants by the human population (*2*), little is known about their natural function in plants. It is known, however, that many plants produce endogenous substances which can discourage insect feeding. These include specific toxins, compounds with pheromone-like activity, and bitter-tasting aversive substances (*3*). This study presents evidence that the methylxanthines have pestistatic and pesticidal activity and describes the biochemical mechanisms by which such activity may occur.

In most experiments, tobacco hornworm (*Manduca sexta*) larvae were used to study pestistatic and pesticidal effects,