plement the flow mechanisms. Both the vascular system and the perineal muscle system may have feedback connections with cavernosal smooth muscle, and contraction of smooth muscle may be the final necessary condition for achievement and maintenance of maximum penile rigidity.

Sleep-related erections are entirely natural events, and because they occur predictably and reliably in all healthy men, they are always available for study. Their duration (an average of 30 minutes per episode in young adults) is comparable to that of sexual erections and allows ample time for dynamic studies of the erectile process. The subject, being asleep, is presumably less conscious of the social and psychological factors that may inhibit erections elicited with erotic stimuli in a laboratory setting. This methodology, including particularly blood flow measurement, holds promise not only for studies of the phylogeny (in some species) and ontogeny of the mechanisms of erection, but also for explorations of erectile pathophysiology and for screening of drugs. Numerous drugs commonly used by psychiatrists and general practitioners are claimed to have stimulating or inhibiting effects on erection (*13*). Since our analyses of inter- and intrasubject variability in circumference change and blood flow showed the sensitivity of the parameters as dependent variables, this new methodology offers a means of systematically examining such claims.

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12. Data for each run (data for about 40 minutes) were initially scanned for the largest values of circumference change and peak-to-trough blood flow. The system was then calibrated as follows: baseline, ground; largest circumference change, 1 V; largest peak-to-trough blood flow pulse, within ±1 V. The basic sampling epoch was 2 seconds for both channels. For the blood flow channel, initial sampling was at a minimum rate of 500 μsec; for each 2-second epoch, the absolute minimum and the absolute maximum voltages were detected and the peak-to-trough voltage was calculated. Finally, the scorers' detections of transient artifacts were used to delete from the data file all 2-second epochs containing such artifacts.
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Light and Agonists Alter Pineal *N*-Acetyltransferase Induction by Vasoactive Intestinal Polypeptide

Abstract. *Vasoactive intestinal polypeptide stimulated serotonin* N-*acetyltransferase activity in rat pineal glands in organ culture by a postsynaptic action that was independent of the beta-receptor. The magnitude of stimulation could be altered by environmental lighting conditions and by prior exposure to the agonist. Such up- and down-regulation, well known for catecholaminergic stimulation of this system, is compatible with a possible control of the pineal by vasoactive intestinal polypeptide as well as by catecholamines.*

Many peptide hormones discovered in peripheral organs also occur in neurons (*1*), are released during neural stimulation (*2*), and affect postsynaptic receptors (*3*). Some appear to exist in the nerve terminal with "classical" neurotransmitters and are released concurrently with them (*4*). Such corelease might modulate the time course or magnitude of the postsynaptic response to the transmitters.

The pineal gland may be useful in examining peptide-transmitter interactions. It is well established that norepinephrine released from sympathetic nerve terminals innervating the gland activates a β_1-adrenergic receptor, elevating adenylate cyclase activity, the concentration of adenosine 3′,5′-monophosphate, and the activity of serotonin *N*-acetyltransferase (NAT) (E.C.2.3.1.5) (*5*). The magnitude of this enzymatic response can be modulated by prior stimulation history: glands deprived of stimulation become supersensitive to agonists while those stimulated for long periods become subsensitive. This homeostatic mechanism of "up- and down-regulation" is common to many physiologically functioning systems and, in the case of catecholaminergic stimulation of the pineal gland, has been shown to be related to the number of titratable receptors (*6*).

Vasoactive intestinal polypeptide (VIP) can also elicit an increase in NAT activity (*7, 8*) by a postsynaptic action, and the pineal contains nerve fibers with immunologically detectable VIP (*9*). The action of VIP on NAT activity seems independent of the beta-receptor, but may involve a peptide receptor. Response to VIP can be blocked by the polypeptide secretin, which shares some peptide sequences with VIP, but not by somatostatin, which does not (*8*). It was of interest to determine whether stimula-

Table 1. Effects of lighting on the pineal response to VIP. Young (120-g) male rats were maintained under a light-dark cycle or exposed to constant light for 4 days. All animals were killed 3 hours after the end of the final dark interval, and their pineals were removed and placed in organ culture (*10*). After 30 minutes 10 μl of water alone, water containing VIP (to a final concentration of 0.01 μ*M*), or isoproterenol (ISO) (to a final concentration of 0.1 μ*M*) was added. Incubation continued for 5 hours. Pineals were then frozen in solid CO_2 and assayed for NAT activity the next day (*10*). Values are means ± standard errors for pineals from six animals. All the values for the "light-dark" group differ significantly from the corresponding values for constant light ($P < .02$, Student's *t*-test).

Additions	NAT activity (nanomoles of product per hour per gland)	
	Constant light	Light-dark
Water	3.00 ± 0.74	0.13 ± 0.01
VIP (0.01 μ*M*)	7.14 ± 0.92	1.74 ± 0.23
ISO (0.1 μ*M*)	25.49 ± 2.77	9.22 ± 0.99
VIP − water*	4.13 ± 0.92	1.61 ± 0.23

*Mean value for water only subtracted from individual values for VIP exposure.

tion can alter the response of the pineal to VIP, as it does the response to catecholamines. The effects of various lighting conditions and of exposure to catecholaminergic agonists were therefore examined.

Young male Fischer 333 rats were maintained for several weeks under a photoperiodic cycle with 10 hours of light and 14 hours of darkness (lights on from 1800 to 0400 hours; light intensity at cage level, 30 footcandles). The rats were killed 3 hours after the final dark period, when pineal sensitivity to catecholaminergic agonists should have been low. A second matched group of animals exposed to constant light for 4 days (90 footcandles at cage level) was killed at the same time. Such exposure should have rendered their pineals supersensitive to catecholamines.

The pineal glands of these animals were removed, placed in organ culture (*10*) for 30 minutes, and then challenged with the beta-receptor agonist isoproterenol (0.1 μM), with VIP (0.01 μM), or with water vehicle alone. The glands were removed after 5 hours, frozen on solid CO_2, and assayed for NAT activity (*10*) the following day. As shown in Table 1, isoproterenol and VIP elicited greater NAT activity in pineals from animals kept under constant light than in those from animals exposed to the light-dark cycle. Even glands treated with water alone showed such a difference, presumably reflecting altered pinealocyte sensitivity to transmitter spontaneously released from the injured nerve. After correction for this baseline shift, NAT activity in pineals from animals exposed to constant light was three times greater than that in pineals from animals exposed to the light-dark cycle.

In a separate experiment, pineals were taken from six animals 3 hours after lights on and from a second group of six animals 9 hours after lights on. The pineals were cultured for 5 hours in medium (*10*) made 2 mM in the β-adrenergic blocker DL-propranolol and 1 μM in VIP. Mean NAT activity (± standard errors) for pineals removed from the rats exposed to 3 hours of light was 2.2 ± 0.4 nmole of product per hour per gland, compared to 4.5 ± 0.8 for the animals exposed for 9 hours ($P < .02$, Student's t-test).

Next the effect of prolonged exposure of cultured pineals to VIP on the response to a subsequent VIP challenge was examined. Despite problems in demonstrating isoproterenol down-regulation under these conditions, an isoproterenol treatment group was included as a control and to see whether sustained stimulation by one agonist might influence the response to another. Pineals from young male rats were treated for 48 hours with isoproterenol (10 μM), VIP (10 μM), or a comparable volume of the water vehicle. The pineals were then washed in a larger volume of fresh, agonist-free medium for 45 minutes to remove trapped agonist and to permit NAT activity to decline and then transferred to fresh medium and exposed to water or agonist for an additional 4 hours.

As expected, isoproterenol-treated glands transferred to agonist-free medium (group H) still had 66 percent of the activity of pineals stimulated with isoproterenol but not previously exposed to this agonist (group E), while pineals treated first with VIP and then with water (group D) showed one-tenth the activity of pineals freshly challenged with VIP (group A) (Table 2). Also as expected, the response to a second isoproterenol treatment (group F) was not significantly different from the response to a single isoproterenol treatment following exposure to water (group E). In contrast, treatment with VIP decreased the response to a subsequent VIP treatment (group B; compare group A), indicating down-regulation by sustained exposure to VIP. Prior treatment with VIP did not, however, affect the response to a subsequent isoproterenol treatment (group G; compare group E), indicating that the apparent down-regulation by VIP is not secondary to nonspecific toxicity accompanying prolonged exposure to VIP. However, treatment with isoproterenol did significantly reduce the response to a subsequent VIP challenge (group C; compare group A). This may be due to a limitation in some essential component (such as adenosine triphosphate) after long-term stimulation by isoproterenol, or it may indicate that a common step is involved in both stimulations. Whether this one-way inhibition has physiological significance remains to be determined.

Table 2. Effect of exposure to catecholaminergic agonists on the response of pineals in organ culture to subsequent exposure. Pineals from young male rats were cultured in medium containing penicillin and streptomycin (*10*) and either VIP (10 μM) or isoproterenol (10 μM). The medium was changed daily. After 48 hours of culture the pineals were placed in fresh medium without agonist for 90 minutes, then transferred to medium containing agonist or water and incubated for four more hours. Values are means ± standard errors (the values in parentheses give the number of pineals). The Newman-Keuls test was used to test for significant differences. The results for groups B, C, and D differ from the result for group A, and B and C differ from D ($P < .02$). Groups E and G differ from H ($P < .05$), but F does not differ significantly from E, G, or H. In a similar experiment with comparable culture conditions, the activity of untreated pineals was 0.28 ± 0.05 nmole of product per hour per gland.

Group	First treatment	Second treatment	NAT activity (nanomoles of product per hour per gland)
		VIP challenge	
A	None	VIP	11.0 ± 1.2 (13)
B	VIP	VIP	6.4 ± 1.3 (13)
C	ISO	VIP	5.8 ± 0.9 (13)
D	VIP	None	1.1 ± 0.2 (11)
		ISO challenge	
E	None	ISO	13.8 ± 1.6 (13)
F	ISO	ISO	11.5 ± 1.6 (12)
G	VIP	ISO	13.8 ± 3.4 (13)
H	ISO	None	7.6 ± 1.0 (13)

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