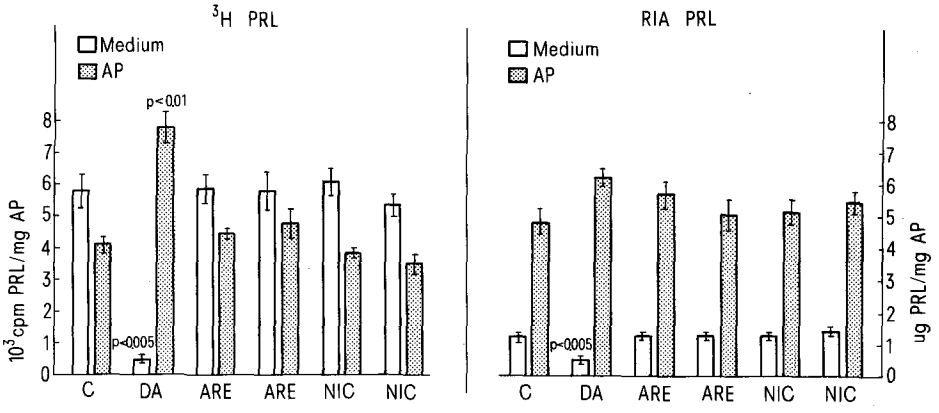

Fig. 2. Effect of dopamine (10^{-6} M), arecoline (10^{-5} and 10^{-4} M) and nicotine (10^{-5} and 10^{-4} M) on prolactin (PRL) secretion by anterior pituitary (AP) explants incubated for 4 h. Each group contained 6 vials and each vial contained 6 AP fragments representing 1 AP equivalent. The incorporation of 4,5-^{3}H-leucine into prolactin is presented on the left side while RIA prolactin is presented on the right side. Each bar represents the mean ± SEM. C, control; DA, dopamine; ARE, arecoline; NIC, nicotine.

did not alter the in vitro prolactin production when measured by either ^{3}H leucine incorporation into prolactin or RIA (figures 1 and 2).

Discussion. We have shown here that the cholinergic agonists arecoline (muscarinic), nicotine (nicotinic) and carbacol (mixed) have little effect on the prolactin production by incubated anterior pituitary explants. These data are in contrast to those of Vale et al.[5] who reported that carbacol, at concentrations of 100 µM (10^{-4} M) significantly inhibited the in vitro secretion of prolactin. These authors further reported that the inhibitory effects of carbacol on prolactin release could be blocked by the addition of atropine to the culture medium. Our inability to confirm the results of Vale et al.[5] was not due to the insensitivity of our explant system since a low dose of dopamine was able to markedly suppress prolactin release. Further, we have shown that 2 other cholinergic agonists, arecoline and nicotine were also unable to suppress prolactin production in vitro. On the basis of this evidence we conclude that cholinergic agonists do not have a direct effect on the pituitary to suppress prolactin secretion.

We have observed previously[9] that the ability of arecoline to suppress the afternoon surge of prolactin was blocked by both atropine sulfate and atropine methyl nitrate. Since the latter drug penetrates the blood brain barrier poorly we suggested that actions of cholinergic agonists are outside the blood brain barrier, perhaps at the level of the pituitary. Alternatively, the drugs may act at the level of the median eminence, which is also outside the blood brain barrier. The data we have reported here indicate that cholinergic agonists do not have a direct action on the pituitary and we therefore conclude that the action may be on the medium eminence to stimulate the release of dopamine. This suggestion is supported by the observation that cholinergic agonists could not suppress the elevated prolactin induced by drugs that block dopamine synthesis or release[10].

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Daily change in pineal N-acetyltransferase activity in a diurnal mammal, the ground squirrel[1]

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Summary. Pineal N-acetyltransferase (NAT) activity in the ground squirrel, a diurnal mammal, was found to have a daily fluctuation with peak activity during the dark time. This same daily change is found in nocturnal mammals and diurnal birds. NAT may play an important role in keeping track of light and dark cycles.

In the mammalian pineal gland, serotonin N-acetyltransferase (NAT) is believed to be the regulatory enzyme in the conversion of serotonin to melatonin. In nocturnal rats, hamsters, gerbils, and guinea pigs[2,3], NAT activity has been shown to have a daily fluctuation with its nadir during the light-time and its peak during the dark time. In diurnal birds (chickens, sparrows, and quail)[4,5], NAT activity was likewise found to be low during light and high during dark times. NAT has not previously been studied in a diurnal mammal such as the ground squirrel.

24 young adult (200-250 g) ground squirrels (*Citellus mexicanus*) were obtained from Otto Martin Locke (New Braunfels, Texas). Animals were kept in 12 h light/12 h dark, lights on at 07.00 h, 2 per cage, for 4 weeks to ensure entrainment to the lighting schedule (figure, A). At each of 12 time points over a 24-h period (August), 2 animals were

Comparison of light and dark-time pineal NAT activity in nocturnal and diurnal animals

Locomotor activity	Species (reference)	N-acetyltransferase activity (nmoles/pineal gland/h) Serotonin substrate Light-time	Serotonin substrate Dark-time	Tryptamine substrate Light-time	Tryptamine substrate Dark-time	Light/dark cycle
Diurnal mammal	Ground squirrel	0.53±0.18	2.75±1.09			12/12
Nocturnal mammals	Albino rat[2,4]	0.18±0.04	2.80±0.23			12/12
	Albino rat[3]			0.5	8.5	14/10
	Albino rat[11]			0.16±0.02	22.8±1.34	14/10
	Guinea pig[3]			0.7	1.0	14/10
	Hamster[4]	0.31±0.04	0.38±0.07			12/12
	Hamster[3]			0.15	0.55	14/10
	Gerbil[3]			0.12	0.28	14/10
Diurnal birds	Chicken[4]	1.75±0.18	47.07±4.86			12/12
	Sparrow[4]	0.18±0.04	3.76±0.89			12/12
	Quail[5], females	1.19±0.19	2.14±0.29			12/12
	Quail[12], females			0.06	0.09	16/8
	Quail[5], males	0.6±0.07	1.43±0.23			12/12
	Quail[12], males			0.07	0.11	16/8
Human	Human[13,14]	3.90±0.51	4.32±1.00	*0.72±0.05	* 1.28±0.10	-

There are 2 assays for NAT activity which differ in terms of substrate utilized and the quantitative value for NAT activity that is obtained. SE are indicated for the data where available. Some data were taken from graphs[3] or converted from pmoles/15 min per 1/7 pineal given by Preslock[12]. All light-time NAT activities were lower than dark-time NAT activities ($p<0.05$) except for the hamsters[4] and humans[13]. *nmoles/h/mg protein.

killed by decapitation. Pineal glands were immediately dissected and frozen on dry ice. The procedure for assay of NAT[6] was modified to increase its sensitivity by adding twice the normal amount of ^{14}C-serotonin to the substrate mixture. Data were analyzed by Student's t-test for difference between mean light and dark values. During housing in 12 h light/12 h dark, some of the squirrels were monitored for locomotor activity using 12 inch activity wheels and an Esterline Angus event recorder[7].

NAT activity in the diurnal ground squirrel (figure, B) has a daily fluctuation with its peak during the dark time. This change is similar to the pattern seen in nocturnal mammals and diurnal birds. The 24-fold change (from minimum light to maximum dark values) is greater in the ground squirrel than in any mammal studied except the rat (table). In making comparisons among the species, a number of factors should be kept in mind: a) the amplitude of the NAT change is affected by photoperiod[8,9] with the finding that the amplitude is greater in nocturnal mammals in long photoperiods (such as 14 h light/10 h dark) than for shorter photoperiods (such as 12 h light/12 h dark); b) the tryptamine assay gives greater quantitative NAT activity for rats than the serotonin assay, but not for quail (table). c) variation in results within a species (e.g. rats, table) are obtained in different laboratories.

N-acetyltransferase activity is high in the dark part of the light-dark cycle in mammals and birds regardless of whether their locomotor behavior is nocturnal or diurnal. This is unusual because other endocrine glands (adrenal and pituitary) have daily changes where the time of peak activity is different correlating with the locomotor activity of the species.

It is known that the pineal gland participates in photoperiodic regulation of reproduction[10]. The occurrence of peak pineal NAT activity during dark irrespective of temporal organization supports the idea that the pineal gland (specifically NAT) may be important in keeping track of light and dark, especially for the seasonal regulation of the reproductive system.

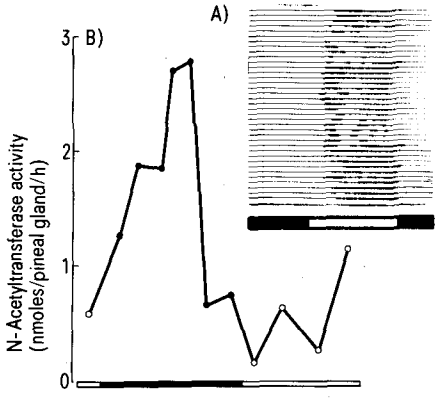

Fig. A. Entrainment of the diurnal locomotor activity of one of the experimental ground squirrels by a light-dark cycle. 12 h light/12 h dark. Dark part of the bar indicates lights out. The ordinate is days. The abscissa is 24 h.

Fig. B. Pineal N-acetyltransferase activity of the ground squirrel (each point is an average for 2 glands). The abscissa is 24 h. Dark part of bar indicates time of lights out (12 h light/12 h dark): ○, light time; ●, dark time. Mean light values differ from mean dark values, $p<0.05$.

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