1 W.B. Quay and J.T. Bagnara, Archs Pharmacodyn. *150*, 137 (1964).
2 J.T. Bagnara, Gen. Comp. Endocr. *3*, 86 (1963).
3 J.T. Bagnara and M.E. Hadley, Am. Zool. *10*, 201 (1970).
4 F.W. Pehlemann, Zool. Anz., suppl., *29*, 571 (1967).
5 P.C. Baker and K.M. Hoff, Comp. gen. Pharmac. *2*, 59 (1971).
6 P.D. Nieuwhoop and J. Faber, in: Normal Table of Xenopus laevis. North Holland Publishing Company, Amsterdam 1956.
7 P.C. Baker, Comp. Biochem. Physiol. *28*, 1387 (1969).
8 P.C. Baker, W.B. Quay and J. Axelrod, Life Sci. *4*, 1981 (1965).
9 P.C. Baker, K.M. Hoff and R.L. Clise, Comp. gen. Pharmac. *2*, 397 (1971).
10 G.A. Buznikov, in: Comparative Pharmacology, vol. I, p. 593. Ed. M.J. Michelson. Pergamon Press, Oxford 1973.
11 W.B. Quay, Adv. Pharmac. *6A*, 283 (1968).

The lack of an effect of cholinergic agonists on anterior pituitary prolactin production in vitro[1]

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Summary. The addition of dopamine to anterior pituitary incubations resulted in a marked decrease (88% for ^{3}H prolactin and 69% for RIA prolactin) in prolactin release. Incubation with the cholinergic agonists carbacol, arecoline and nicotine resulted in no significant change in prolactin secretion.

The role of the cholinergic system in the regulation of prolactin secretion is not well understood. Subramanian and Gala[3] have shown that cholinergic agonists can inhibit the afternoon surge of prolactin when administered systemically to ovariectomized estrogen-treated rats. Lawson and Gala[4], however, have shown that systemically administered cholinergic agonists had little effect on basal prolactin levels in ovariectomized estrogen-treated rats. Vale et al.[5] have suggested that the pituitary may have cholinergic receptors since the addition of carbacol to anterior pituitary cell cultures and explants resulted in an inhibition of prolactin secretion. The purpose of the present study was to determine the effects of a muscarinic agonist, arecoline, a nicotinic agonist, nicotine, and carbacol, a mixed cholinergic agonist on prolactin secretion by anterior pituitary (AP) explants.

Materials and methods. Adult female Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Mich.) weighing approximately 250 g were housed 2 per cage, and placed on a 14:10 h lighting schedule. All animals had free access to water and Purina Rat Chow. Approximately 5 days later animals were decapitated, the AP quickly excised, and dissected into 4 or 6 pieces. An AP fragment from each pituitary was placed in each group so as to permit statistical analysis with a paired t-test. Each experiment had a total of either 4 or 6 vials per group and each vial contained 1 AP equivalent. Pituitaries were incubated as described previously by MacLeod and Lehmeyer[6] with the following modifications. The AP were preincubated for 2 h in 1 ml Hanks' balanced salt solution containing 10 µCi 4,5-^{3}H-leucine/ml. The pituitaries were removed, rinsed with Medium 199 and incubated in Medium 199 for 4 h under a gas environment of 95% O_2-5% CO_2. The incubation medium contained one of the following drugs: dopamine (1.0×10^{-6} M), carbacol (1.0×10^{-5} and 1.0×10^{-4} M), arecoline (1.0×10^{-5} and 1.0×10^{-4} M) and nicotine (1.0×10^{-5} and 1.0×10^{-4} M). At the end of 4 h the AP were removed from the incubation medium and homogenized in 3 ml of a 1% Triton X-100-PBS solution. The resulting solution was centrifuged; the supernatant and the incubation medium were frozen for future assay. The prolactin concentration of the medium and the AP homogenates was determined using a rat prolactin RIA as previously described[7]. The incorporation of 4,5-^{3}H-leucine into prolactin was determined by disc gel electrophoresis[8]. 200 µl of incubation medium and AP homogenate solution were assayed for prolactin using a 7.5% polyacrylamide gel. The stained band was cut from the gel, dissolved in 0.5 ml of NCS solution and the radioactivity counted in a Packard tri-carb spectrophotometer. Statistical analysis of the data was accomplished using the paired t-test and the data is presented as the average ± SEM.

Results. As shown in figures 1 and 2, the addition of dopamine (10^{-6} M) to the incubation medium resulted in a decrease in prolactin release of 88% and 69% as measured by ^{3}H-leucine incorporation into prolactin and RIA, respectively. The decrease in medium prolactin was reflected by an increase in pituitary content for both methods of assay but only ^{3}H prolactin was statistically significant. The addition of cholinergic agonists to the incubation medium

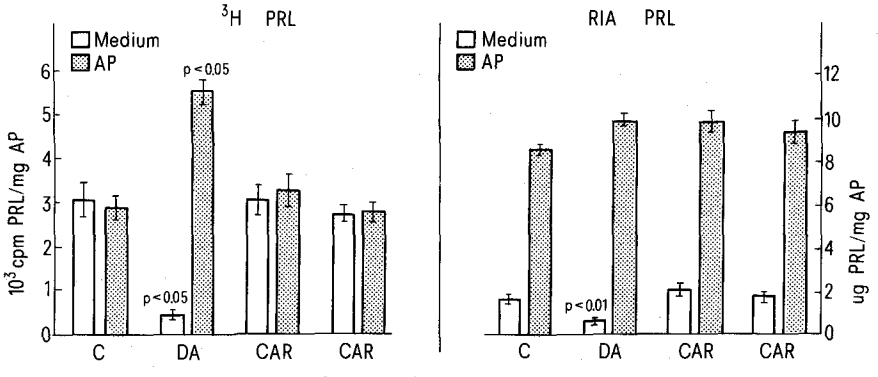

Fig. 1. Effect of dopamine (10^{-6} M) and carbacol (10^{-5} and 10^{-4} M) on prolactin (PRL) secretion by anterior pituitary (AP) explants incubated for 4 h. Each group contained 4 vials and each vial contained 4 fragments representing 1 AP equivalent. The incorporation of 4,5-^{3}H-leucine into prolactin 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; CAR, carbacol.

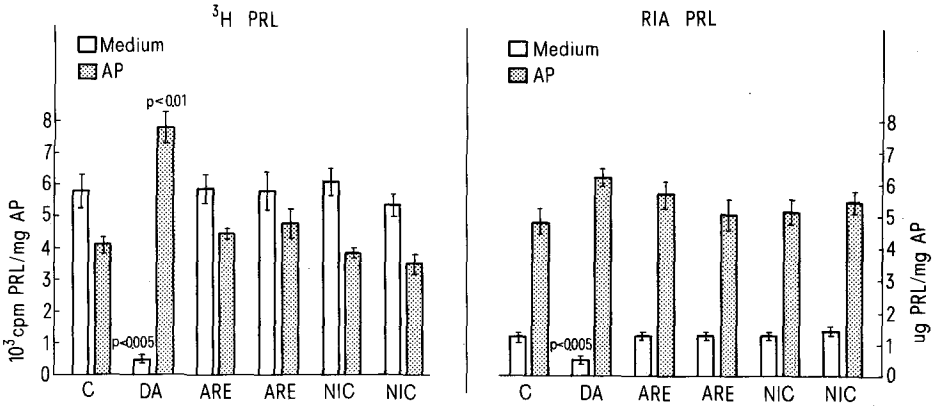

Fig. 2. Effect of dopamine (10^{-6} M), arecoline (10^{-5} and 10^{-4} M) and nicotine (10^{-5} and 10^{-4} M) on prolactin (PRL) secretion by anterior pituitary (AP) explants incubated for 4 h. Each group contained 6 vials and each vial contained 6 AP fragments representing 1 AP equivalent. The incorporation 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].

1 Supported in part by NSF Research grant No. BMS 74-17332.
2 The authors appreciate receiving as a gift from the National Institute of Arthritis, Metabolic and Digestive Diseases, the rat prolactin used for iodination (RP-I_2) and standards (RP-1).
3 M.G. Subramanian and R.R. Gala, Endocrinology *98*, 842 (1976).
4 D.M. Lawson and R.R. Gala, Endocrinology *96*, 313 (1975).
5 W. Vale, C. Rivier, M. Brown, L. Chan, N. Ling and J. Rivier, in: Hypothalamus and Endocrine Functions, p.397. Ed. F. Labrie, J. Meites, and G. Pelletier. Plenum Press, New York 1976.
6 R.M. MacLeod and J.E. Lehmeyer, Endocrinology *94*, 1077 (1974).
7 E.Y.H. Kuo and R.R. Gala, Biochim. biophys. Acta *264*, 462 (1972).
8 R.M. MacLeod and A. Abad, Endocrinology *83*, 799 (1968).
9 M.G. Subramanian and R.R. Gala, Proc. Soc. exp. Biol. Med. *155*, 353 (1977).
10 L. Grandison and J. Meites, Endocrinology *99*, 775 (1976).

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