PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 155, 353-356 (1977)

Specificity of Arecoline and Apomorphine and the Site of Action of Arecoline in Inhibiting the Diurnal Prolactin Surge[1] (39805)

MARAPPA G. SUBRAMANIAN AND RICHARD R. GALA[2]

Department of Physiology, Wayne State University School of Medicine, Detroit, Michigan 48201

The influence of adrenergic, serotonergic, and cholinergic amine systems on the afternoon surge of plasma prolactin in ovariectomized, polyestradiol phosphate (PEP)-injected rats has been reported by us (1-3). Administration of cholinergic blockers did not have any effect on this diurnal surge of prolactin (1, 2), however, atropine sulfate (AS) was capable of blocking the inhibitory effect of arecoline (2). Recent studies have raised the question of specificity for some receptor blockers. Atropine was shown to prevent the α-receptor blockade of adrenergic blockers (4), and methiothepin could block both serotonergic and dopaminergic receptors (5, 6). Studies by us (7) and by others (8) suggested the presence of cholinergic components outside the blood brain barrier that could inhibit prolactin secretion.

The aim of the present study was to examine the effects of mecamylamine (a nicotonic blocker) and atropine methylnitrate (AMN; a muscarinic blocker with low permeability across the blood brain barrier) on the arecoline (a muscarinic agonist)-induced inhibition of the diurnal prolactin surge. The specificity of apomorphine, which was shown to inhibit prolactin release in basal (9-12), in diurnal surge (13), and in pituitary culture studies (13, 14), was also examined by determining whether AS could block the inhibitory effect of apomorphine on the diurnal surge.

Materials and methods. Adult female Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Mich.) 200 to 225 g in body weight were ovariectomized and maintained under a 14:10 light-dark schedule at 23 ± 2°. Animals were fed laboratory rat chow and given free access to water. When they were 275 to 300 g in body weight, an aortic catheter was inserted according to the procedure of Lawson and Gala (15) and the animals were given a single subcutaneous injection of 1 mg of Estradurin (Ayerst), which is equivalent to 0.5 mg of PEP. Blood sampling was initiated 7 to 9 days after catheterization and estrogen administration. The experimental protocol was as described in detail previously (1).

The drugs used in this study were administered intra-arterially through the catheter and were as follows: arecoline HCl (Pfaltz and Bauer, Inc., Flushing, N.Y.), atropine methylnitrate and atropine sulfate (Sigma, St. Louis, Mo.), mecamylamine HCl (a gift from Dr. Horace D. Brown, Merck Sharp & Dohme Research Laboratories, Rahway, N.J.), and apomorphine HCl (Merck Sharp & Dohme, Rahway, N.J.). All drugs were dissolved in 0.9% saline except apomorphine which was dissolved in 0.1% sodium metabisulfite. Drug dosages were based on our previous studies (1, 2, 10) and the amounts administered were as the derivatives supplied by the manufacturer. In experiments in which combination of a blocker and an agonist was used, the blocker was always administered 5 min before the agonist. The injection schedule and the dosages for the drugs are given in the legends of the figures.

Plasma prolactin was assayed by the double-antibody radioimmunoassay at two dilutions each in duplicate (16). Prolactin values are expressed in nanograms per milliliter of plasma in terms of NIAMDD rat prolactin RP-1 standard (11 IU/mg). Statistical comparisons were made (i) within each group (the 1300-, 1500-, 1700-, 1900-, and 2100-hr values were individually compared against the 1100-hr values) to determine the occurrence of the afternoon surge, and (ii) between the control and experimental

[1] Supported by Research Grant BMS-74-17332 from the National Science Foundation.

[2] To whom reprint requests should be sent.

ISSN 0037-9727

groups (the prolactin values of the experimental group at the same time of the day were compared against the control group) to evaluate the magnitude of the afternoon surge, using Student's *t* test.

Results. In the control group, a sharp rise in plasma prolactin was observed at 1500 hr, reached the peak at 1700 hr, and then decreased continuously at 1900 hr and 2100 hr (Figs. 1-3). Administration of AMN at 1200 hr and every 2 hr thereafter did not significantly alter the pattern of the afternoon surge. The muscarinic agonist, arecoline, blocked the prolactin surge. Administration of AMN before arecoline overcame the inhibitory effect of arecoline and resulted in a statistically significant rise in plasma prolactin at 1700, 1900, and 2100 hr (Fig. 1). The amount of AMN administered (5.3 mg/kg at 1200 hr and 2.65 mg/kg every 2 hr thereafter) was equivalent to 10 and 5.0 mg/kg of AS, in terms of atropine base. Administration of mecamylamine did not have any effect on the prolactin surge or

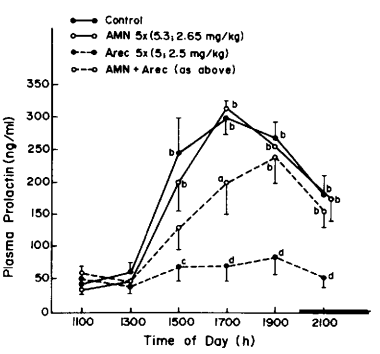

FIG. 1. The influence of atropine methylnitrate (AMN) on the arecoline HCl (Arec)-induced inhibition of the prolactin afternoon surge in ovariectomized, Estradurin (1 mg)-injected rats. ●——●, Control (six animals); O——O, injected with AMN, 5.3 mg/kg at 1200 hr and 2.65 mg/kg at 1400, 1600, 1800, and 2000 hr (eight animals); ●-----●, Arec, 5 mg/kg at 1200 hr and 2.5 mg/kg at 1400, 1600, 1800, and 2000 hr (five animals); O-----O, AMN plus Arec (five animals). All drugs in this and subsequent experiments were administered intra-arterially through the catheter. Vertical lines represent the standard errors of the mean. Statistical comparisons were made within each group, 1300-, 1500-, 1700-, 1900-, and 2100-hr values against 1100-hr values (a = $P < 0.05$, b = $P < 0.01$) and between control and experimental groups at the same time of the day (c = $P < 0.05$, d = $P < 0.01$).

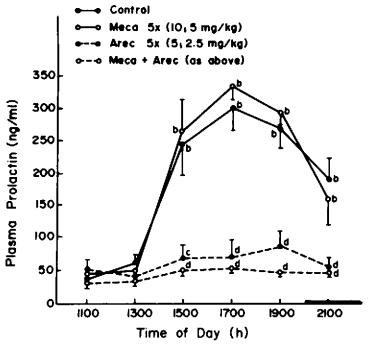

FIG. 2. The influence of mecamylamine HCl (Meca) on the arecoline HCl (Arec)-induced inhibition of the prolactin afternoon surge in ovariectomized, Estradurin (1 mg)-injected rats. ●——●, Control (six animals); O——O, injected with Meca, 10 mg/kg at 1200 hr and 5 mg/kg at 1400, 1600, 1800, and 2000 hr (five animals); ●-----●, Arec, 5 mg/kg at 1200 hr and 2.5 mg/kg at 1400, 1600, 1800, and 2000 hr (five animals); O-----O, Meca plus Arec (five animals). Vertical lines represent the standard errors of the mean. Statistical comparisons were made as in Fig. 1.

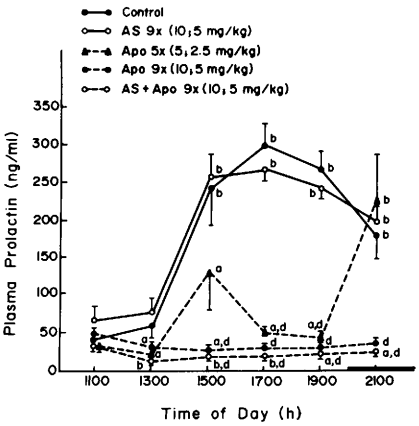

FIG. 3. The influence of atropine sulfate (AS) on the apomorphine HCl (Apo)-induced inhibition of the prolactin afternoon surge in ovariectomized, Estradurin (1 mg)-injected rats. ●-----●, Control (six animals); O——O, injected with AS, 10 mg/kg at 1200 hr and 5 mg/kg every hour thereafter (eight animals); ▲-----▲, Apo, 5 mg/kg at 1200 hr and 2.5 mg/kg at 1400, 1600, 1800, and 2000 hr (five animals); ●-----●, Apo, 10 mg/kg at 1200 hr and 5 mg/kg every hour thereafter (five animals); O-----O, AS plus Apo, both 10 mg/kg at 1200 hr and 5 mg/kg every hour thereafter (five animals). Vertical lines represent the standard errors of the mean. Statistical comparisons were made as in Fig. 1.

on the arecoline-induced suppression of the prolactin surge (Fig. 2).

Apomorphine, when administered at 5 mg/kg at 1200 hr and 2.5 mg/kg every 2 hr thereafter, did not completely block the surge and, in fact, at 2100 hr the level was comparable to that of control. When apomorphine was given at a dose of 10 mg/kg at 1200 hr and 5 mg/kg every hour thereafter, not only was the surge blocked but there was also a significant inhibition of the plasma prolactin level at 1300 and 1500 hr when compared to the 1100-hr level (Fig. 3). AS at a dose of 10 mg/kg at 1200 hr and 5 mg/kg every hour thereafter had no effect on the plasma prolactin surge and did not overcome or attenuate the apomorphine-induced inhibition of the prolactin surge (Fig. 3).

The administration of the drugs resulted in specific behavioral responses. Following each arecoline injection the animals exhibited tremors (a CNS effect) and extensive salivation (a peripheral effect). Administration of AMN prior to arecoline blocked the peripheral effect but not the central effect. The rats injected with mecamylamine prior to arecoline exhibited both salivation and tremors. Following apomorphine administration, either alone or in combination with AS, the animals showed stereotyped behavioral responses such as continuous gnawing, licking, biting, and increased aggressiveness.

Discussion. The observations from this study and our previous studies (1, 2) indicate that cholinergic stimulation blocked the diurnal surge of plasma prolactin in ovariectomized, estrogen-treated rats. It has been suggested that AMN has poor permeability across the blood brain barrier (17, 18). In the present study this concept is confirmed by the observation that AMN administered prior to arecoline blocked the peripheral response (salivation) but not the central response (tremors) evoked by arecoline. We have observed that the administration of AS prior to arecoline blocked both the tremors and salivation (Subramanian and Gala, unpublished) and that these animals exhibited a prolactin surge similar to that of the controls (2). Our observation here that AMN was able to overcome, especially in the later time periods, the inhibitory effects of arecoline on the prolactin surge becomes even more significant when viewed in the context of the behavioral response. The results obtained from this study and the potentiating effects of both AS and AMN on the perphenazine-induced prolactin release in monkeys (7) lend credence to the hypothesis that there are cholinergic receptors outside the blood brain barrier inhibitory to prolactin release. Such a cholinergic component could very well be located on the pituitary itself, as suggested by Vale and co-workers (19). These latter investigators demonstrated that cholinergic agonists inhibited prolactin release from cultured pituitary cells and that atropine could overcome this effect. The observation that mecamylamine did not have any effect on the arecoline-induced inhibition of prolactin surge and behavioral effects demonstrated the absence of an overlapping effect of nicotinic blockers on the muscarinic receptors.

Since the administration of cholinergic blockers did not modify the time of onset, magnitude, or decline of the diurnal prolactin surge, it was suggested that the cholinergic system does not normally operate to inhibit prolactin release in this phenomenon (2). However, the inhibitory effect on prolactin release following the activation of the cholinergic system, and the existence of cholinergic receptors in the pituitary, suggest that the cholinergic system might play a role under certain conditions to inhibit prolactin release. The diurnal prolactin surge investigated in the present and previous studies (1, 2) is apparently not one of these phenomena.

The administration of apomorphine every 2 hr did not result in complete blockade of the surge. This is probably due to the short effect apomorphine has on suppressing prolactin release, which has been observed by us (10) and by others (12, 20). However, apomorphine administration every hour starting at 1200 hr, either alone or in combination with AS, resulted in a complete blockade of the surge. Stereotyped behavioral responses, such as continuous nondiscrete gnawing, sniffing, licking, biting, chewing, and increased aggressiveness (21–23), were observed following each administration of apomorphine either alone or with AS and indicated that AS does not block

dopaminergic receptors in the CNS.

Summary. Ovariectomized, polyestradiol phosphate (PEP)-injected, catheterized rats exhibiting a daily diurnal plasma prolactin surge were used to investigate the specificity of cholinergic receptor blocker drugs and to demonstrate the presence of cholinergic receptors outside the blood brain barrier. Atropine methylnitrate (AMN) blocked the inhibitory effect of arecoline on the diurnal surge of plasma prolactin and this blockade was more dramatic for the later time periods. The tremorogenic effect of arecoline (a CNS action) was not blocked, whereas the salivation effect of arecoline (a peripheral action) was blocked by AMN. The prolactin and behavioral responses were taken to indicate that AMN does not pass the blood brain barrier and that there are cholinergic receptors inhibiting prolactin release outside this barrier, perhaps directly on the pituitary. Mecamylamine, a nicotine blocker, did not have any effect either on the arecoline-induced blockade of the prolactin surge or on the arecoline-induced behavioral responses. Atropine sulfate (AS), a muscarinic blocker, did not influence either the apomorphine-induced inhibition of the prolactin surge or the apomorphine-induced behavioral responses.

We would like to express our appreciation to Mrs. Cynthia Van De Walle for her expert technical assistance in prolactin radioimmunoassay and excellent art work. We also appreciate receiving as a gift the rat prolactin used for iodination and standards from NIAMDD Rat Pituitary Hormone Distribution Program.

1. Subramanian, M. G., and Gala, R. R., Endocrinology **98,** 842 (1976).
2. Subramanian, M. G., and Gala, R. R., Neuroendocrinology, in press.
3. Subramanian, M. G., and Gala, R. R., IRCS Med. Sci. **4,** 529 (1976).
4. Kapur, H., and Mottram, D. R., Pharmacol. Res. Commun. **7,** 463 (1976).
5. Monachon, M.-A., Burkhard, W. P., Jalfre, M., and Haefely, W., Naunyn-Schmiedebergs Arch. Pharmakol. **274,** 192 (1972).
6. Lawson, D. M., and Gala, R. R., Endocrinology **98,** 42 (1976).
7. Gala, R. R., Subramanian, M. G., Peters, J. A., and Jaques, S., Jr., Horm. Res. **7,** 118 (1976).
8. Donoso, A. O., and Bacha, J. C., J. Neural Transm. **37,** 155 (1975).
9. Subramanian, M. G., Lawson, D. M., and Gala, R. R., Life Sci. **18,** 305 (1976).
10. Lawson, D. M., and Gala, R. R., Endocrinology **96,** 313 (1975).
11. Brown, W. A., Res. Commun. Chem. Pathol. Pharmacol. **11,** 671 (1975).
12. Horowski, R., Newmann, F., and Graf, K. J., J. Pharm. Pharmacol. **27,** 532 (1975).
13. Smalstig, E. B., Sawyer, B. D., and Clemens, J. A., Endocrinology **95,** 123 (1974).
14. MacLeod, R. M., and Lehmeyer, J. E., Endocrinology **94,** 1077 (1974).
15. Lawson, D. M., and Gala, R. R., J. Endocrinol. **62,** 75 (1974).
16. Kuo, E. Y. H., and Gala, R. R., Biochim. Biophys. Acta **264,** 462 (1972).
17. Innes, I. R., and Nickerson, M., *in* "The Pharmacological Basis of Therapeutics." (L. S. Goodman and A. Gilman, eds.), p. 524. Macmillan Co., New York (1970).
18. Libertun, C., and McCann, S. M., Proc. Soc. Exp. Biol. Med. **147,** 498 (1974).
19. Vale, W., Rivier, C., Brown, M. Chan, L., Ling, N., and Rivier, J. *in* "Hypothalamus and Endocrine Functions." (F. Labrie, J. Meites, and G. Pelletier, eds.), p. 397, Plenum Press, New York (1976).
20. Mueller, G. P., Simpkins, J., Meites, J., and Moore, K. E., Neuroendocrinology **20,** 121 (1976).
21. Gale, K. N., Murray, J. P., and Horita, A., Proc. West Pharmacol. Soc. **18,** 375 (1975).
22. Poignant, J. C., Gressier, H., Petitjean, M., Regneir, G., and Canevari, R., Experientia **31,** 1204 (1976).
23. Sourkes, T. L., Psychoneuroendocrinology **1,** 69 (1975).

Received January 14, 1977. P.S.E.B.M. 1977, Vol. 155.