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The Influence of Adrenalectomy and of Corticosterone Administration on the Ether-Induced Increase in Plasma Prolactin in Ovariectomized Estrogen-Treated Rats[1] (40066)

MARAPPA G. SUBRAMANIAN AND RICHARD R. GALA

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

We have reported that various anesthetics release prolactin in ovariectomized (OVX) and OVX–estrogen treated rats (1–3). A number of investigators have observed that prolactin and corticosterone are both released by suckling (4–7) and stressful conditions (6–9). Other investigators have indicated that the administration of dexamethasone, a synthetic glucocorticoid, will attenuate the ether-induced (10, 11) and thyrotropin releasing hormone (TRH)-induced (12) prolactin release. The present study was undertaken to examine (a) the effect of continuous ether anesthesia for 2 hr on prolactin release in adrenalectomized animals and (b) the influence of exogenously administered corticosterone, the species-specific glucocorticoid in the rat, on ether-induced prolactin release.

Materials and methods. Female Sprague–Dawley rats (Spartan Research Animals, Inc., Hasslett, MI) were ovariectomized (OVX) when 200–225 g in body weight and housed under controlled temperature (23±2°) and artificial illumination (14 h light and 10 h darkness). When they were about 275 g in body weight an aortic catheter was inserted as described in detail previously (1) and the rats were given a single subcutaneous injection of 1 mg Estradurin (equal to 0.5 mg of polyestradiol phosphate). Bilateral adrenalectomies (ADX) were performed 3 days after catheterization and ADX animals were maintained on 0.9% NaCl. Serial blood sampling was initiated 8 days after catheterization and the experimental protocol was as described previously (3). Briefly, between 08:00 hr and 09:00 hr on the day of sampling, a polyethylene extension filled with saline and fitted witha three-way stop cock was attached to the indwelling catheter and the animals were left undisturbed for at least 60 min. After this equilibration period an initial blood sample (0.6 ml) was withdrawn (time 0). In the control group subsequent blood samples were obtained at 10, 30, 60, and 120 min. In the experimental groups, after the 0 min sample, the animals were anesthetized in a large jar saturated with ether vapor. In those animals receiving corticosterone, a single bolus of the hormone was administered intraarterially through the catheter after the 0 min sample. Complete anesthesia was achieved within about 2 min, and the rats were maintained under ether for the duration of the experiment using a nose cone. Additional blood samples were obtained at 10, 30, 60, and 120 min after the initiation of anesthesia. After each blood sample the volume was immediately replaced with sterile saline at 37°. The blood samples were diluted with an equal volume of chilled phosphate buffer saline (pH 7.6) and centrifuged at 3°. The plasma was separated and stored at −20°. Plasma prolactin levels were assayed by the double antibody radioimmunoassay (13) at two dilutions each in duplicate. Rat prolactin NIAMD-RP-1 with a potency of 11 IU/mg was the standard. Plasma corticosterone levels were determined by competitive binding assay (14) in duplicate at a single dilution. Statistical comparisons were made within a group using the analysis of variance and Duncan multiple range test and between treatment groups using a two-way analysis of variance with computor assistance (15).

Results. The plasma prolactin values are shown in Table I. The plasma prolactin levels in the control animals were not significantly altered by the sampling procedure. In the OVX–Estradurin-injected animals (OVX–E_2) under ether, there was a significant rise in plasma prolactin in 10 min ($P<0.01$), followed by a decrease to a slightly higher level than the initial value and this level was maintained for the remainder of the experiment

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(120 min). The OVX–ADX–E_2 animals under ether were observed to have an increase in plasma prolactin for all time periods when compared with the initial level. The administration of corticosterone (500 μg or 25 μg/rat i.a.) completely block the initial ether-induced prolactin increase (10 min); however, at subsequent time periods there was a gradual increase in plasma prolactin levels to that of adrenalectomized animals (120 min; Groups IV and V vs III).

The plasma corticosterone levels are presented in Table II. In the OVX–E_2 animals under ether, plasma corticosterone at 30, 60, and 120 min were significantly higher than the initial 0 min value. The OVX–ADX–E_2–corticosterone (500 μg/rat) administered animals had extremely high plasma corticosterone level at 10 min which progressively declined in subsequent periods. Those animals injected with 25 μg/rat of corticosterone showed a similar trend but with a less pronounced increase in plasma corticosterone at 10 min.

Discussion. In estrogenized animals a rise and decline in plasma prolactin following ether anesthesia has been observed in this study and in our previous studies (2, 3). This pattern of prolactin release was not restricted to only ether but has been observed with other anesthetics such as methoxyflurane (3) and with restraint stress (16). It has been reported by others that the prolactin increase to ether stress (10, 11) and TRH administration (12) could be attenuated by dexamethasone. Further, it has been shown that in situations like suckling, plasma corticosterone increased in conjunction with prolactin (7). The plasma prolactin pattern of a sustained increase under ether in ADX animals and a sharp rise and fall in adrenal intact animals suggests that, under ether, the fall in prolactin after the initial increase may be due to the inhibitory feedback action of the elevated corticosterone. This hypothesis was further strengthened by the results obtained in this study. Here following exogenous administration of corticosterone there was no increase in plasma prolactin following ether anesthesia; however, as the exogenous corticosterone was metabolized from circulation, the plasma prolactin level increased. Although previous studies with dexamethasone suggested a negative feedback (10–12), the use of corticosterone, which is a natural glucocorticoid, lends further credence to the hypothesis that adrenal corticoids have an inhibitory effect on prolactin secretion in rats. This inhibitory feedback action, however, does not appear applicable to all conditions of increased prolactin release since in suckling, where prolactin release is more pronounced and sustained (6, 17) than during ether exposure (6), and where there is also a simultaneous increase in plasma corticosterone levels (7), little to no decrease in plasma prolactin is observed. This suggests that in rats there may be a different mechanism between suckling and ether in the induction of prolactin release.

Summary. Ovariectomized, polyestradiol-phosphate (PEP) injected, catheterized rats subjected to continuous ether anesthesia

TABLE I. EFFECTS OF ETHER ANESTHESIA ON PLASMA PROLACTIN LEVELS.

Group	Treatment	Plasma prolactin levels (ng/ml)**				
		0 min	10 min	30 min	60 min	120 min
I	Control OVX + E_2 (7)*	59.5 ± 11.1	50.7 ± 5.3	60.4 ± 8.4	48.5 ± 7.8	55.6 ± 4.6
				under ether anesthesia		
II	OVX +E_2 + Ether (7)	44.5 ± 3.6	108.2 ± 17.1[b]	68.1 ± 10.5	64.7 ± 15.7	67.2 ± 14.2
III	OVX + ADX + E_2 + Ether (15)	46.0 ± 4.3	120.3 ± 23.5[a]	109.0 ± 18.6[a]	125.7 ± 22.7[b]	111.9 ± 21.0[a]
IV	OVX + ADX + E_2 + B 500 μg/rat + Ether (10)	43.0 ± 2.8	64.1 ± 14.6	74.2 ± 9.7	87.9 ± 15.9	99.3 ± 25.0
V	OVX + ADX + E_2 + B 25 μg/rat + Ether (5)	44.4 ± 6.9	42.4 ± 3.9	95.6 ± 20.4	59.0 ± 6.3	126.4 ± 43.0

* The number in parentheses represents the number of animals in each group. Statistical comparisons were made using analysis of variance and comparisons of each time period were made to the 0 min values within each experimental group using Duncan's multiple range test (a = $P < 0.05$, b = $P < 0.01$). Using two-way analysis of variance the following group comparisons for treatments were made: I vs II, NS; II vs III, $P < 0.05$; III vs IV $P < 0.05$; III vs V, NS. OVX = ovariectomy; ADX = adrenalectomy; E_2 = polyestradiol phosphate; B = corticosterone.

** Mean ± SE.

TABLE II. EFFECTS OF ETHER ANESTHESIA ON PLASMA CORTICOSTERONE LEVELS.

Group	Treatment	Plasma corticosterone levels (μg/100 ml)**				
		0 min	10 min	30 min	60 min	120 min
I	Control OVX + E_2 (7)*	34.8 ± 5.4	45.2 ± 5.8	50.5 ± 3.5	50.2 ± 10.4	36.2 ± 8.5
				under ether anesthesia		
II	OVX + E_2 + Ether (7)	34.0 ± 3.7	53.4 ± 6.7	59.7 ± 7.7[a]	63.4 ± 6.6[b]	62.7 ± 8.4[a]
III	OVX + ADX + E_2 + B 500 μg/rat + Ether (10)	6.3 ± 0.6	2110.0 ± 224.0[b]	202.3 ± 16.8[b]	56.7 ± 8.6[b]	18.3 ± 3.5[b]
IV	OVX + ADX + E_2 + B 25 μg/rat + Ether (5)	6.0 ± 0.5	161.0 ± 24.7[b]	26.0 ± 4.3[b]	7.6 ± 0.4	5.8 ± 0.6

* The number in parentheses represents the number of animals in each group.

** Mean ± SE.

Statistical comparisons were made using analysis of variance and comparisons of each time period were made to the 0 min value within each experimental group using Duncan's multiple range test (a = $P < 0.05$; b = $P < 0.01$). Using two-way analysis of variance the following group comparisons for treatments were made: I vs II, $P < 0.05$. OVX = ovariectomy; ADX = adrenalectomy; E_2 = polyestradiol phosphate; B = corticosterone.

showed an increase in plasma prolactin level at 10 min followed by lower levels at 30, 60, and 120 min. Plasma corticosterone levels were increased significantly under continuous ether anesthesia. In contrast to intact animal, bilaterally adrenalectomized animals under ether anesthesia showed a sustained elevation of plasma prolactin at all time periods. In adrenalectomized animals subjected to ether anesthesia the exogenous administration of corticosterone (either 500 μg/rat or 25 μg/rat) prevented the increase in plasma prolactin at 10 min period when plasma level of corticosterone was high. In subsequent periods as the plasma corticosterone decreased, the plasma prolactin increased. It is suggested that corticosterone has an inhibitory feedback effect on the ether-induced prolactin release.

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1. Lawson, D. M., and Gala, R. R., J. Endocrinol. **62,** 75 (1974).
2. Lawson, D. M., and Gala, R. R., J. Endocrinol. **66,** 151 (1975).
3. Subramanian, M. G., Lawson, D. M., and Gala, R. R., Life Sci. **18,** 305 (1976).
4. Voogt, J. L., Sar, M., and Meites, J., Amer. J. Physiol. **216,** 655 (1969).
5. Amenomori, Y., Chen, C. L., and Meites, J., Endocrinology **86,** 506 (1975).
6. Terkel, J., Blake, C. A., and Sawyer, C. H., Endocrinology **91,** 49 (1972).
7. Stern, J. M., and Voogt, J. L., Neuroendocrinology **13,** 173 (1974).
8. Neill, J. D., Endocrinology **87,** 1192 (1970).
9. Ajika, K., Kalra, S. P., Fawcett, C. P., Krulich, L., and McCann, S. M., Endocrinology **90,** 707 (1972).
10. Euker, J. S., Meites, J., and Riegle, G. D., Endocrinology **96,** 85 (1975).
11. Harms, P. G., Langlier, P., and McCann, S. M., Endocrinology **96,** 475 (1975).
12. Schwinn, G., Von Zur Muhlen, A., and Warnecke, U., Acta Endocrinol. **82,** 486 (1976).
13. Kuo, E. Y. H., and Gala, R. R., Biochim. Biophys. Acta **264,** 462 (1972).
14. Sparagana, M., and Lucera, L., Biochemical Med. **9,** 363 (1974).
15. Steel, R. G. D., and Torrie, J. H., Principles and Procedures of Statistics, McGraw Hill, New York, p. 99 (1960).
16. Smith, S. W., and Gala, R. R., J. Endocrinol. **74,** 303 (1977).
17. Subramanian, M. G., and Reece, R. P., Proc. Soc. Exp. Biol. Med. **149,** 754 (1975).

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