PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 162, 191–193 (1979)

Anterior Pituitary Enzyme Activities and Prolactin Secretion in Male Rats Treated with Psychotropic Drugs (40644)[1]

IVAN NAGY, CARLOS A. VALDENEGRO, IVAN S. LOGIN, AND ROBERT M. MACLEOD

Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22908

Estradiol has been shown to influence hypothalamic catecholamine turnover (1, 2) and to stimulate prolactin secretion directly (3, 4). Recently we reported that estrogen administration also caused increased pituitary glycolytic enzyme activity which may be associated with the increased hormone synthesis (5). These studies showed that estrogen feedback regulates lactotrophic metabolic activity as well as hormone production. Psychotropic drugs increase prolactin secretion either by altering hypothalamic catecholamine metabolism or by inhibiting their binding to the lactotrophic membrane receptors (3, 6). In the present experiments we studied the effect of using catecholamine antagonists to manipulate prolactin release on pituitary hexose-monophosphate shunt, glycolytic, oxidative, and lysosomal enzyme activities.

Materials and methods. Mature, male, Sprague–Dawley rats housed in a constant temperature room (22–23°) under artificial illumination (0600–1800 hr) received 1.0 mg perphenazine (Schering Company, Bloomfield, N.J.), 0.2 mg pimozide (Janssen Pharmaceuticals, Beerse, Belgium), or 0.5 mg reserpine (Serpasil, 2.5 mg/ml, CIBA Pharmaceutical Company, Summit, N.J.) s.c. daily for 3 days. Twenty-four hours after the third injection the animals were decapitated. Trunk blood was collected for prolactin radioimmunoassay, and enzyme activities and protein concentration in anterior pituitary tissue were determined as previously reported (7). The metabolic pathways were characterized by the following enzymes: The hexose-monophosphate shunt by D-glucose-6-phosphate, NADP oxidoreductase, EC 1.1.1.49 (G6P-DH); glycolysis by ATP, pyruvate 2-*o*-phosphotransferase, EC 2.7.1.40 (PK), and by L-lactate, NAD oxidoreductase, EC 1.1.1.27 (LDH); citric acid cycle by L-malate, NAD oxidoreductase, EC 1.1.1.37 (MDH), and by threo-D_s-isocitrate, NADP oxidoreductase, EC 1.1.1.42 (ICDH); and lysosomal breakdown by orthophosphoric monoester phosphohydrolase, EC 3.1.3.2 (tartrate-inhibited acid phosphatase).

Results. The injection of rats with psychotropic drugs produced serum prolactin levels which were increased 400% after perphenazine, 200% after pimozide, and 70% after reserpine treatment (Fig. 1).

Neuroleptic treatment did not change anterior pituitary weight significantly (Table I). The water-soluble protein concentration of the gland increased by 10–15%; the change was significant after perphenazine and pimozide treatment.

Enzyme activities (Tables II and III) are expressed as activity per milligram pituitary tissue, activity per milligram protein, and activity per pituitary gland. Perphenazine and reserpine did not affect G6P-DH activity, while pimozide increased its pituitary concentration and content slightly but significantly. Psychotropic drugs did not affect PK activity (mU/mg gland and mU/mg protein); after pimozide treatment the slightly enlarged glands contained more of this enzyme. LDH activity (mU/mg gland) elevated slightly only after perphenazine treatment. Perphenazine decreased ICDH activity (on all three bases) moderately; ICDH activity (mU/mg protein) also decreased after reserpine administration. MDH- and tartarate-inhibited acid phosphatase activities were not influenced by the treatments.

Discussion. It is well documented that catecholamines inhibit prolactin secretion and that their antagonists stimulate it. Increased synthesis of the hormone has also been reported following treatment with neuroleptics (3, 6). The present experiments showed a clear increase in serum prolactin concentra-

[1] This work was supported by USPHS Research Grant CA-07535-15 from the National Cancer Institute.

0037-9727/79/090191-03$01.00/0

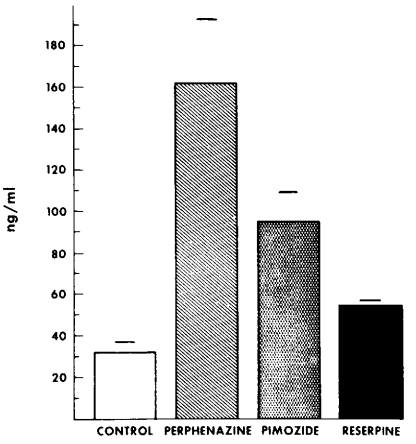

FIG. 1. Serum prolactin concentration of male rats treated with psychotropic drugs. The hormone concentration elevated significantly ($P < 0.01$) in each group after drug administration. All values are mean ± SEM. Injection schedule as in Table I.

TABLE I. EFFECT OF PSYCHOTROPIC DRUGS ON ANTERIOR PITUITARY WEIGHT AND PROTEIN CONTENT IN MALE RATS

Treatment	No. of animals	Anterior pituitary (mg)	Protein μg/mg gland	Protein μg/ gland
Control	8	7.63	91.3	699.0
		±0.26	±5.9	±51.1
Perphenazine	7	7.32	108.5[a]	791.6
		±0.24	± 4.3	±33.9
Pimozide	8	8.39	104.3	872.7[a]
		±0.31	±4.9	±46.9
Reserpine	7	7.06	108.1	754.6
		±0.31	±5.3	±24.8

[a] $P < 0.05$ versus controls. All values are mean ± SEM. Perphenazine 1.0 mg, pimozide 0.2 mg, and reserpine 0.5 mg were injected s.c. daily for 3 consecutive days. The animals were killed 24 hr after the last injection..

tions in male rats after perphenazine, pimozide, or reserpine treatment, while pituitary water-soluble protein concentration, oxidative enzyme activity, and tartrate-inhibited acid phosphatase activity remained unchanged.

Estradiol also increases prolactin secretion, primarily and directly stimulating synthesis of the hormone, which in turn causes increased prolactin release (3, 4, 8). In addition to its direct pituitary influence, estradiol inhibits dopamine release into the hypophyseal portal blood (1, 2), further stimulating release of the hormone. However, unlike the neuroleptics, which have no observable effect on lactotroph enzyme metabolism, estradiol causes increased glycolytic enzyme activity which appears to be associated with the estradiol-induced increases in prolactin synthesis (9).

The association of prolactin synthesis and enzyme activity has been observed in other systems as well. Rats bearing transplantable mammotropic pituitary tumors have atrophied pituitary glands with decreased prolactin synthesis (3). The administration of bromocriptine (3, 8) and of antiestrogens (10) to rats produces similar effects. In these cases, pituitary hexosemonophosphate pathway and anaerobic glycolytic activities also decreased significantly (7, 10). Numerous other morphological, physiological, and biochemical evidences support the hypothesis that total enzyme activities measured in pituitary homogenates reflect the metabolic activity of

TABLE II. ACTIVITY OF PITUITARY GLAND ENZYMES IN THE ANTERIOR PITUITARY OF MALE RATS TREATED WITH PSYCHOTROPIC DRUGS

Treatment	mU/mg gland	mU/mg protein	mU/ gland
Glucose-6-phosphate dehydrogenase			
Control	3.329	36.74	25.60
	±0.166	±1.23	±1.76
Perphenazine	3.654	33.78	26.72
	±0.128	±0.78	±1.29
Pimozide	4.066[a]	38.92	33.99[b]
	±0.199	±1.52	±1.78
Reserpine	3.666	33.76	25.64
	±0.242	±1.18	±1.66
Pyruvate kinase			
Control	18.98	200.2	145.0
	±2.78	±17.6	±21.4
Perphenazine	20.85	185.7	151.7
	±1.96	±14.0	±14.4
Pimozide	24.64	231.2	205.6[a]
	±2.21	±13.5	±18.4
Reserpine	21.50	197.6	150.3
	±1.66	±9.5	±11.1
Lactate dehydrogenase			
Control	57.2	645.2	438.8
	±1.2	±45.8	±21.8
Perphenazine	62.1	579.2	453.8
	±1.3[a]	±27.4	±14.2
Pimozide	58.2	564.5	487.0
	±1.4	±35.4	±16.4
Reserpine	55.9	525.5	394.6
	±1.0	±30.7	±19.5

[a] $P < 0.05$ versus controls. All values are mean ± SEM. Injection schedule as in Table I.

[b] $P < 0.01$ versus controls.

TABLE III. ACTIVITY OF PITUITARY GLAND ENZYMES IN THE ANTERIOR PITUITARY OF MALE RATS TREATED WITH PSYCHOTROPIC-DRUGS

Treatment	mU/mg gland	mU/mg protein	mU/gland
Isocitrate dehydrogenase			
Control	2.200 ±0.123	25.28 ±2.86	16.90 ±1.23
Perphenazine	1.811[a] ±0.049	16.97[a] ±1.20	13.26[a] ±0.58
Pimozide	2.052 ±0.137	20.06 ±2.09	16.98 ±0.79
Reserpine	1.872 ±0.113	16.74[a] ±1.27	14.18 ±1.32
Malate dehydrogenase			
Control	277.5 ±7.5	3,148.4 ±260.2	2,130.3 ±114.0
Perphenazine	279.7 ±6.8	2,621.3 ±183.0	2,045.4 ±82.3
Pimozide	264.1 ±5.5	2,577.9 ±206.2	2,206.9 ±59.7
Reserpine	285.8 ±7.5	2,685.5 ±161.1	2,014.7 ±102.0
Tartarate-inhibited acid phosphatase			
Control	0.532 ±0.025	5.93 ±0.35	4.08 ±0.26
Perphenazine	0.558 ±0.021	5.20 ±0.29	4.06 ±0.11
Pimozide	0.508 ±0.033	4.91 ±0.37	4.23 ±0.25
Resperine	0.576 ±0.021	5.40 ±0.36	4.08 ±0.27

[a] $P < 0.05$ versus controls. All values are mean ± SEM. Injection schedule as in Table I.

the lactotrophs (summarized in (5)).

We previously suggested that estradiol primarily stimulates prolactin biosynthesis, which increases promptly following administration of the drug (11). The demands of increased prolactin biosynthesis for newly synthesized glycolytic enzymes presumably activate the glycolytic metabolism of the lactotrophs (5). By contrast, the psychotropic drugs primarily stimulate prolactin release. Following their injection the gland releases previously synthesized hormone, greatly depleting its prolactin stores. The depletion may exert a positive intracellular feedback on prolactin biosynthesis (4, 12). New synthesis of mRNA or preprolactin have been observed (13), but subchronic psychotropic administration failed to stimulate the synthesis of newly formed glycolytic enzymes.

Hence these experiments suggest strongly that estrogen and neuroleptics stimulate prolactin synthesis through different mechanisms and that prolactin and enzyme synthesis have different gene loci in the lactotrophs, which can be stimulated by estrogen but not by the exclusion of the monoamines. While chronic administration of dopamine antagonists might stimulate the glycolytic metabolism of lactotrophs, that would represent a pharmacological situation far removed from physiological circumstances.

Summary. Psychotropic dopamine antagonists primarily stimulate prolactin release, and their subchronic administration does not influence the water-soluble protein concentration, the hexosemonophosphate shunt, and anaerobic glycolytic, oxidative, or lysosomal activity of the lactotrophs.

1. Ben-Jonathan, N., Oliver, C., Weiner, H. J., Mical, R. S., and Porter, H. J., Endocrinology **100,** 452 (1977).
2. Cramer, O. M., Parker, C. R., Jr., and Porter, J. C., Endocrinology **104,** 419 (1979).
3. MacLeod, R. M., *in* "Frontiers in Neuroendocrinology" (L. Martini and W. F. Ganong, eds.), Vol. 4, p. 169. Raven Press, New York (1976).
4. Meites, J., Lu, K. H., Wuttke, W., Welsch, C. W., Nagasawa, H., and Quadri, S. K., *in* "Recent Progress in Hormone Research" (E. B. Astwood, ed.), Vol. 28, p. 471. Academic Press, New York (1972).
5. Nagy, I., and MacLeod, R. M., submitted for publication.
6. MacLeod, R. M., and Login, I. S., *in* "Cellular Mechanisms Modulating Gonadal Hormone Action" (R. L. Singhal and J. A. Thomas, eds.), Vol. 2, p. 211. Univ. Park Press, Baltimore (1976).
7. Nagy, I., and MacLeod, R. M., Mol. Cell. Endocrinol. **13,** 317 (1979).
8. MacLeod, R. M., and Nagy, I., Fed. Proc. **38,** 1025 (1979).
9. Nagy, I., Hirka, G., Kurcz, M., Anda, E., and Baranyai, P., Enodkrinologie **71,** 1 (1978).
10. Nagy, I., Valdenegro, C. A., Login, I. S., and MacLeod, R. M., Abst. Endocr. Soc. 61st Annu. Meet. **116** (1979).
11. MacLeod, R. M., and Lamberts, S. W. J., *in* "Proceedings of the International Symposium on Neuroendocrine Correlates in Neurology and Psychiatry" (E. Müller and A. Agnoli, eds.), p. 89. Elsevier/North-Holland, Amsterdam (1979).
12. Sulman, F. G., *in* "Hypothalamic Control of Lactation" (F. G. Sulman, ed.), p. 88. Springer-Verlag, New York (1970).
13. Stone, R. T., Maurer, R. A., and Gorski, J., Biochemistry **16,** 4915 (1977).

Received April 30, 1979. P.S.E.B.M. 1979, Vol. 162.