Effect of Perphenazine on the Secretion of Prolactin *in Vivo* and *in Vitro*[1] (36960)

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Antipsychotic tranquilizers such as chlorpromazine and reserpine have been reported to cause persistent lactation when given to rats (1), rabbits (2) and humans (3). Recently, injection of chlorpromazine, acepromazine or perphenazine has been found to cause a rise in plasma prolactin (PRL) levels in rats (4), sheep (5), goats (6) and humans (7), as measured by radioimmunoassay or bioassay.

It has been suggested that one of these drugs, perphenazine, allows plasma PRL levels to rise by inhibiting the release of a PRL (release) inhibiting factor (PIF) from the hypothalamus. This conclusion was based on the observation that treatment of rats with perphenazine for 10 days lowered hypothalamic "PIF activity" as determined by coculture of hypothalamic fragments with pituitary glands *in vitro* (8) and by the observation that treatment of rats with perphenazine caused an increase in hypothalamic "PIF content" (9) as determined by prevention of pituitary depletion of PRL content in rats treated with perphenazine (10).

In an effort to understand the mechanism by which the antipsychotic tranquilizers cause the release of PRL, this study was undertaken to define the phenomenon in terms of dose and time, and to determine whether perphenazine affects the release of PRL by acting at the level of the pituitary as studied *in vitro*.

Methods and Results. Animals. Normal male and female rats (150–200 g) were purchased from Holtzman Co., Madison, WI. Hypophysectomized rats (200 g) were obtained from Hormone Assay, Inc., Chicago, IL. All animals were housed 4/cage, fed and watered *ad libitum*, and maintained in an environment of 14 hr light–10 hr darkness. All hypophysectomized rats were given citrus fruit and dextrose–saline to supplement their diet. *Drugs.* Perphenazine (Trilafon), 5 mg/ml was purchased from Schering Co. and was used for the *in vivo* studies. Crystalline perphenazine (N. F.) was furnished courtesy of Dr. Preston Perlman, Schering Co., and was used for the *in vitro* experiments. *Assays.* All animals were anesthetized with ether and a 1 ml blood sample was taken by puncture of the external jugular vein ($t = 0$). Drugs were administered iv or ip immediately following withdrawal of the first blood sample. Second blood samples were obtained by decapitation or venous bleeding. Blood samples were kept cold at all times; they were centrifuged at 1500*g* and the plasma was decanted and frozen for assay.

Primary cultures of dispersed rat pituitary cells were prepared as described by Vale *et al.* (11). The effect of various treatments on the rate of secretion of PRL by these cells represents one of our *in vitro* assays for agents that stimulate or inhibit PRL release. The test period was 4 hr after which time the culture fluids were removed and diluted with 1% BSA and assayed for PRL.

PRL concentrations in plasma and culture fluids were determined by a specific radioimmunoassay as described by Blackwell *et al.* (12). Radioimmunoassay data were analyzed by computer using IMSTAT, an exponential curve fitting program (Amoss *et al.*, unpublished data). Subsequently, data were submitted to statistical analysis using EXBIOL, a multivarient computer program (13).

Experimental Design and Results. I. Male and female rats were injected (ip) with 1 mg

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TABLE I. Effect of Perphenazine on Prolactin Secretion *in Vivo*.

Treatment[a]	*N*	PRL (ng/ml) time: 0	1 hr	
A.				
150 g males	4	19 ± 4	86 ± 8	
180 g females	4	78 ± 13	258 ± 50	
Following injection[b] (min)		PRL (ng/ml) Mean ± SE		p^c
B.				
0	24	35	2	
2.5	4	68	6	NS
5	4	70	10	NS
10	4	126	17	—[d]
15	4	168	12	—[d]
30	4	247	84	—[d]
60	4	117	8	—[d]
Dose[e]				
C.				
Saline	5	<10		
100 ng	5	<10		
1 μg	5	<10		
10 μg	5	110	20	—[d]
100 μg	5	149	19	—[d]
250 μg	5	203	19	—[d]

[a] Dose, 1 mg perphenazine ip.

[b] Dose 250 μg perphenazine ip.

[c] Level of statistical significance of the difference from control as determined by the one-sided multiple comparison test of Dunnett: NS = not significant; [d] 99%.

[e] Various doses of perphenazine given iv.

of perphenazine. After 1 hr plasma PRL levels were found to be increased 4 times in animals of both sexes (Table I). Subsequently, adult male rats were divided into groups of 6 each. A blood sample was taken at $t = 0$. This was followed by iv injection of 250 μg perphenazine. The animals were decapitated at either 2.5, 5, 10, 15, 30, or 60 min postinjection. Plasma PRL levels were significantly increased after 10 min, a maximal response was reached by 30 min, and the levels were decreased by 60 min (Table I). Using a similar experimental design, male rats (150 g) were treated with either saline or 100 ng, 1 μg, 10 μg, 100 μg, or 250 μg of perphenazine and each rat was decapitated 30 min postinjection. The 100 ng and 1 μg doses of perphenazine failed to increase plasma PRL levels, while the 10, 100, or 250 μg doses caused a significant increase in the plasma levels (Table I).

II. Quartered pituitaries (2) taken from adult female donors were transplanted under the kidney capsule of hypophysectomized rats. One month later a 1 ml blood sample was taken as described earlier and 1 mg perphenazine was administered ip in 1 ml saline. A second blood sample was taken by iv puncture 1 hr later. Plasma PRL levels were found to be elevated in all rats tested (Table II). The responses ranged from a low of 7% to a high of 300%. The average response was 72%.

III. Doses ranging from 10 ng to 50 μg perphenazine/ml medium were tested for PRL releasing activity using enzymatically dispersed rat pituitary cells which were incubated for 6 days prior to the beginning of the experiment. Immediately before exposure to the drug, the culture medium was changed. Subsequently, the cells were allowed to incubate with the drug for 4 hr. Treatment of the pituitary cells *in vitro* with doses of 10 and 50 μg perphenazine/ml medium was found to produce detachment of the cells from the culture dishes. Doses of 10, 100, and 1000 ng perphenazine/ml did not cause detachment. None of these doses had a stimulatory effect on the secretion of PRL *in vitro* (Table III).

Discussion. Injection of small doses of

TABLE II. Effect of Perphenazine on Plasma Prolactin Levels in Rats Bearing Pituitary Transplants.

Anim. no.[a]	PRL (ng/ml) $t = 0$	$t = 60$ min	% increase
1	45	50	11
2	45	89	97
3	63	78	27
4	28	30	7
5	28	111	300
6	69	100	45
7	40	55	37
8	26	54	53
Av	43	71	72

[a] All animals received 1 mg of perphenazine (ip) at $t = 0$, 30 days after transplant.

TABLE III. Effect of Perphenazine (P) on the Secretion of Prolactin *in Vitro* by Dispersed Pituitary Cells in Monolayer Cultures.

Treatment	*N*	PRL (ng/ml)	Range
A. Saline	3	819	(838–806)
10 ng P/ml	2	903	(946–860)
100 ng P/ml	2	675	(728–622)
1 μg P/ml	1	936	—
B. Saline	3	114	(154–79)
10 ng P/ml	2	155	(221–88)
100 ng P/ml	2	158	(185–130)
1 μg P/ml	2	146	(153–138)

perphenazine into adult rats has been shown to cause a significant increase in plasma PRL levels within 10 min as measured by radioimmunoassay. This response is not an artifact produced by stress, since it has been observed that plasma PRL levels measured at 2.5 min or longer postether anesthesia and jugular vein exposure are never greater than those levels which are found in samples taken immediately following these operations (Blackwell, unpublished data).

It should be noted that the rate of increase in plasma PRL levels following iv injection of perphenazine is similar to the rate of change seen in plasma LH and TSH levels when luteinizing hormone releasing factor (LRF) or thyrotropin releasing factor (TRF), respectively, are administered by the same route. Therefore, one must consider that PRL release follows a time course similar to that seen when administering releasing factors and one must account for this time function when attempting to explain the mechanism by which perphenazine induces PRL release. This criteria must be satisfied whether one advocates that perphenazine releases a PRF (PRL releasing factor) or inhibits the secretion of a PIF.

Simultaneous (iv) administration of either 50 μg norepinephrine, 50 μg epinephrine, 50 μg dopamine, 50 μg L-dopa, 200 mU lysine vasopressin, 200 mU oxytocin, 10 μg TRF, or 10 μg LRF together with 10 μg perphenazine does not inhibit or potentiate the secretion of PRL 30 min later (Blackwell, unpublished data). This suggests to us that these compounds are neither the PRF nor the PIF that has been reported by others (14, 15) to be present in various hypothalamic extracts.

Whatever mechanism of action is involved in the stimulation of PRL release, it seems probable that perphenazine does not bring about this effect by acting at the level of the pituitary, although we have shown that injection of large doses of perphenazine into rats bearing pituitaries transplanted under the kidney capsule causes a marginal (but significant) elevation in plasma PRL levels. Arimura (16) recently reported a 2- to 3-fold increase in plasma PRL levels following administration of perphenazine to a similar preparation. He concluded that the drug may cause extra hypothalamic effects which alter PRL secretion but offered no specific explanation for his observations. Our data and the data of Arimura might be explained by changes in the circulating levels of some hypothalamic agent, or by the affect of perphenazine on other brain areas.

Our *in vitro* data are consistent with the suggestion that the primary effect of perphenazine is not at the level of the pituitary since doses of 10, 100, and 1000 ng perphenazine/ml medium do not stimulate the secretion of PRL by pituitary cells in culture. Further, we have found that addition of doses of perphenazine greater than 1 μg/ml medium causes cell detachment from the culture dishes. Therefore, any effects on PRL levels might be due to random changes in hormone secretion and are considered physiologically irrelevant. Since we have found (unpublished data) that TRF can stimulate the secretion of PRL using such a system, thus demonstrating that the cells are potentially responsive to other stimuli, this demonstrates that perphenazine does not act directly at the level of the pituitary to bring about rapid release of PRL and implies that it affects PRL secretion by stimulating or inhibiting the release of some brain messenger. The mechanism by which this powerful drug brings about this latter effect remains to be determined.

Summary. Injection (iv) of perphenazine, an antipsychotic tranquilizer, into rats produces a 10-fold increase in plasma prolactin levels as measured by radioimmunoassay; a similar response is seen when using adult

male or female rats; plasma prolactin levels are increased significantly by 10 min post-injection and a maximal response occurs after 30 min. Ten micrograms of perphenazine is the minimal active dose that will cause an elevation in plasma prolactin levels. Addition of up to 10 μg/ml medium of perphenazine to *in vitro* cultures of enzymatically dispersed rat pituitary cells does not stimulate secretion of prolactin. Also (ip) injection of 1 mg perphenazine into rats bearing 2 pituitary transplants under the kidney capsule, causes only a marginal increase in prolactin secretion. These data indicate that perphenazine releases prolactin by acting on brain centers above the level of the pituitary.

1. Talwalker, P., Meites, J., Nicoll, C., and Hopkins, T., Amer. J. Physiol. **199**, 1073 (1960).

2. Meites, J., Proc. Soc. Exp. Biol. Med. **96**, 728 (1957).

3. Winnik, H., and Tennenbaum, L., Presse. Med. **63**, 1092 (1955).

4. Ben-David, M., Danon, A., and Sulman, F., Neuroendocrinology **6**, 336 (1970).

5. Bryant, G., and Greenwood, F. C., J. Endocrinol. **40**, 4 (1968).

6. Bryant, G., Connan, R., and Greenwood, F. C., J. Endocrinol. **41**, 613 (1968).

7. Kleinberg, D., Noel, G., and Frantz, A., J. Clin. Endocrinol. Metab. **33**, 873 (1971).

8. Danon, A., Dikstein, S., and Sulman, F., Proc. Soc. Exp. Biol. Med. **114**, 366 (1963).

9. Danon, A., and Sulman, F., Neuroendocrinology **6**, 295 (1970).

10. Danon, A., Dikstein, S., and Sulman, F., J. Endocrinol. **46**, 237 (1970).

11. Vale, W., Grant, G., Amoss, M., Blackwell, R., and Guillemin, R., Endocrinology, in press.

12. Blackwell, R., Vale, W., Amoss, M., Burgus, R., Monahan, M., Rivier, J., Ling, N., and Guillemin, R., Amer. J. Physiol., in press.

13. Sakiz, E., and Guillemin, R., C. R. Acad. Sci. **254**, 3743 (1962).

14. Nicoll, C., Fiorindo, R., McKennee, C., and Parsons, J., *in* "Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry" (J. Meites, ed.), p. 151. Williams & Wilkins, Baltimore (1970).

15. Kragt, C., and Meites, J., Endocrinology **80**, 1170 (1967).

16. Arimura, A., Wakabayash, I., and Dunn, J., Meet. Endocrine Soc., 53rd (Abstr. 61) 1971.

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