Pharmacology Biochemistry & Behavior, Vol. 24, pp. 67–70, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Avoidance Behavior and Plasma Prolactin Levels in Lergotrile Mesylate Treated Rats[1]

D. B. YELVINGTON, G. K. WEISS AND A. RATNER[2]

Department of Physiology, University of New Mexico School of Medicine, Albuquerque, NM 87131

Received 5 March 1984

YELVINGTON, D. B., G. K. WEISS AND A. RATNER. *Avoidance behavior and plasma prolactin levels in lergotrile mesylate treated rats.* PHARMACOL BIOCHEM BEHAV **24**(1) 67–70, 1986.—We have previously shown that psychological factors play a major role in control of prolactin (PRL) secretion, and that PRL increases during shock-motivated avoidance conditioning. In the present studies, we examined whether we could attenuate acquisition performance by suppressing the PRL increase during avoidance testing. Rats were tested daily in a shuttle box. They were presented with a light stimulation followed by an electric footshock. During each trial, the rats were given the opportunity to escape the footshock by moving to a safe side of the box. Movement to the appropriate location after the warning signal (light) begins, but before the onset of the footshock, constitutes a conditioned avoidance response (CAR). Experimental rats were fitted with an intreperitoneal osmotic minipump which delivered lergotrile mesylate (LM), 0.69 mg/kg/day. Blood samples were collected from an indwelling cannula and analyzed by radioimmunoassay. Administration of LM blocked the PRL increase that occurred during early avoidance testing, but did not alter the acquisition of a CAR. These data do not support the idea that PRL acts to facilitate acquisition of avoidance behavior.

Prolactin Lergotrile mesylate Conditioned avoidance response Avoidance behavior

IT is well known that stress can influence prolactin (PRL) secretion in both humans and lower animals [15, 16, 22, 23]. If one assumes that the stress response aids the organism in resisting the effect of the stressor, then it is reasonable to hypothesize that a stress-induced increase in PRL serves some useful function in terms of facilitating the ability to cope with the stressor.

Recent findings have shown that PRL or a prolactin-like material does exist in the cerebrospinal fluid and brain tissue [13, 20, 25]. There is also evidence that PRL can have biological effects on the CNS including behavioral modifications [8, 9, 21]. One such behavioral modification could be to facilitate the acquisition of a response which would aid the animal in avoiding the stressor. In support of this idea, a recent report provided evidence that hyperprolactinemia could enhance acquisition of an avoidance behavior [10]. The idea that a pituitary hormone can affect the acquisition of a conditioned avoidance response (CAR) is not new. DeWied has shown that ACTH, beta-MSH, and lysine vasopressin all can facilitate acquisition of behavior [7].

In a recent study, we showed that PRL levels in rats increased during the first two days of testing when they were being conditioned to avoid a painful stimulus [27]. Our hypothesis is that the increase in PRL occurring during conditioned avoidance can facilitate acquisition performance. To test this hypothesis, we pharmacologically inhibited the release of PRL during avoidance conditioning by administering lergotrile mesylate (LM), a dopamine agonist, and examined its effect on an adaptive behavioral response.

METHOD

Male Sprague-Dawley rats (400–500 g) obtained from Simonsen Labs (Gilroy, CA) were used in this study. Rats were housed in a climate-controlled area (24±2°C) with a fixed light:dark cycle. Lights were on at 0500 hr and off at 1900 hr. Rats were given food and water ad lib.

Rats were acclimated to the presence of the experimenter by being handled daily for at least 2 weeks prior to experimentation. Animals were fitted with a right atrial cannula at appropriate times and allowed two days to recover from surgery before experiments were performed. During the experiments, blood draws were made by means of a heparinized syringe (15 U/ml saline) which was connected to a polyethylene extension of the indwelling cannula. This allowed the rat free movement and permitted the experimenter to draw blood without disturbing the animal. To ensure that the animals were kept close to an isovolemic state, fluid replacement was made with each blood draw, using Plasmanate™ (Cutter Labs, Berkeley, CA).

All experiments were performed between 1300 and 1730 hr. Blood samples were centrifuged at 3500 rpm for 20 min-

[1]Preliminary reports of this work were presented at the meeting of the American Physiological Society, San Diego, 1982.
[2]Requests for reprints should be addressed to Dr. Albert Ratner.

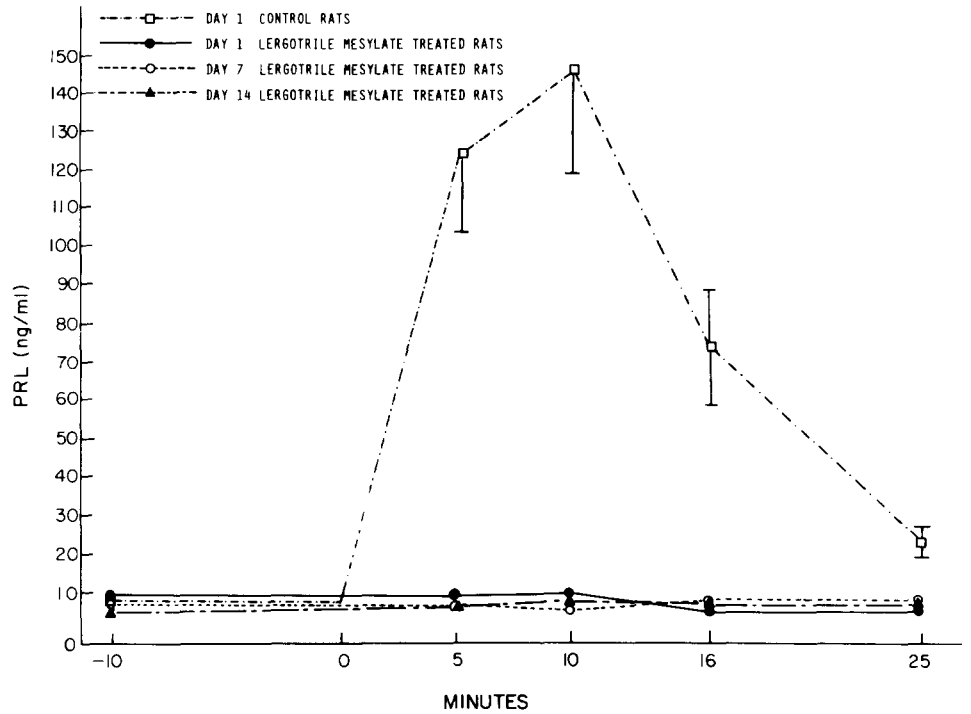

FIG. 1. Plasma prolactin PRL levels during acquisition testing in control and lergotrile mesylate (LM) treated rats. At time zero, rats were placed in a shuttle box and tested for acquisition performance (as described in the Method section). Blood draws were made 10 minutes before and 5, 10, 16, and 25 minutes following placement of the rat in the box on day 1, day 7, and day 14 of testing. LM was delivered to the rats via IP-implanted osmotic minipumps secreting 0.69 mg LM/kg/day. Values indicate mean plasma PRL levels±SEMs (N=7–12).

utes and the plasma separated and stored at −20°C until assayed. Plasma PRL levels were determined in duplicate by radioimmunoassay utilizing a double antibody technique. Rat PRL antibody (rabbit) and rat PRL reference preparations were provided by the Hormone Distribution Program of the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases. Iodine-labeled PRL was obtained from New England Nuclear Corporation (Boston, MA). The limit of sensitivity of the assay was 3 ng/ml and the interassay variation was 9%.

The PRL data were analyzed using the Student-Newman-Keuls procedure with analysis of variance. A p value of less than 0.05 was considered significant.

The Conditioned Avoidance Response (CAR) Paradigm

The CAR paradigm is a procedure that can be used to quantitate the acquisition performance of an avoidance response. The procedure was performed as follows. On the first day of testing, each rat was removed from its home cage and placed in a wooden avoidance box (35 cm long × 25 cm wide × 20 cm high) equipped with a stainless steel grid floor which was used to deliver a scrambled footshock. A 10 watt light bulb was located 10 cm above the grid floor in the center on each end of the box. The animal was allowed one minute to acclimate to the box before a light was turned on. If the rat moved to the opposite side of the box within five seconds of the presentation of the light, the light was turned off and the rat was not shocked. Such a response is termed a conditioned avoidance response (CAR). If, however, the rat did not move to the opposite side of the box within five seconds, the rat was subjected to a 0.3-milliamp scrambled footshock applied across adjacent grids. The current and light remained on until the rat moved to the opposite side of the box or until ten seconds of footshock had elapsed. After one minute (the intertrial interval), the light was once again turned on and the procedure repeated. Ten such trials were presented to the rat on each day of testing over a three-week period. The percentage of CARs performed per day were plotted over time. The data obtained were indicative of the rat's ability to learn and/or perform the avoidance response.

Acquisition Studies Using a Lergotrile Mesylate (LM) Minipump

Two groups of rats were used in this study. The first (controls) were cannulated two days prior to testing and in addition had osmotic minipumps (Alza, Palo Alto, CA) containing saline placed intraperitoneally (IP). Blood draws were made 10 minutes prior to, and 5, 10, 16, and 25 minutes following, the placement of the rat in the testing box on day 1. In a previous study we showed that after one week of testing, PRL levels did not change during acquisition testing [27].

A second group of rats were also cannulated two days prior to the first day of testing, but in addition these rats had osmotic minipumps containing lergotrile mesylate (LM; Eli Lilly, Indianapolis, IN) placed IP. These pumps delivered LM at a calculated dose of 0.69 mg/kg/day. Mills *et al.* [19] have shown that IP placement of LM-containing minipumps can block the PRL response to ether stress. Blood samples were again taken 10 minutes prior to, and 5, 10, 16, and 25 minutes following, the initiation of acquisition testing in LM-treated rats on days 1, 7, and 14 of testing.

Behavioral data concerning the acquisition performance

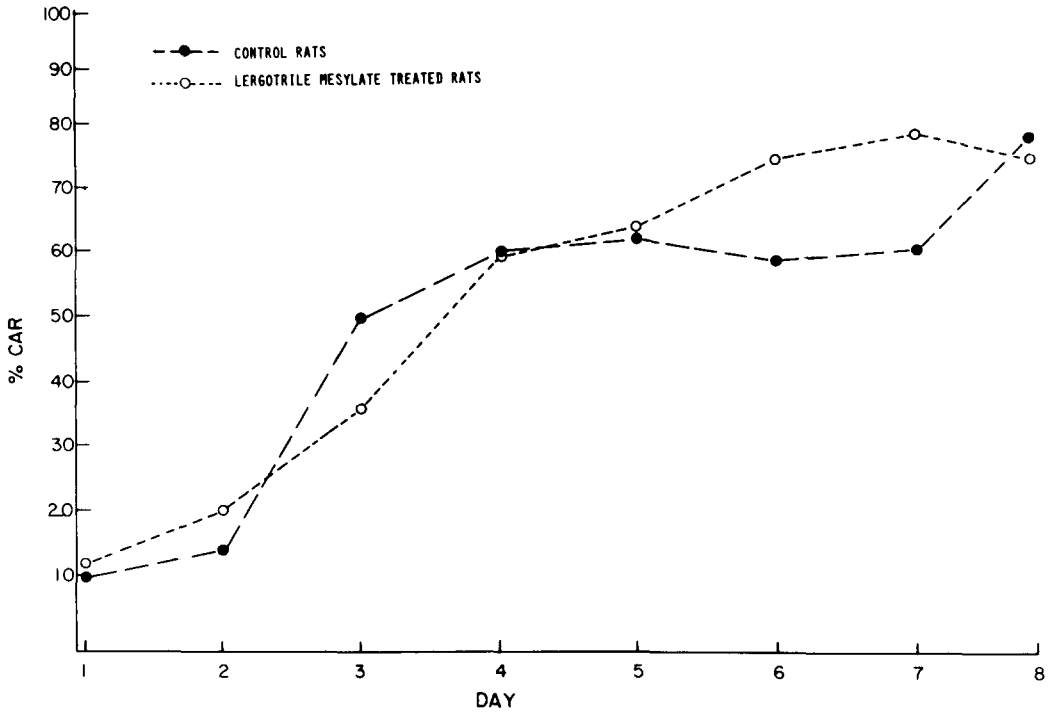

FIG. 2. The acquisition of avoidance behavior during the first 8 days of training. Rats were tested daily in a shuttle box (as described in the Method section). LM was delivered to the rats via IP-implanted osmotic minipumps secreting 0.69 mg LM/kg/day. Values are given as mean % conditioned avoidance responses (CARs) (N=7–10).

of CARs were also obtained each day over a 3-week period in both control and LM-treated rats.

The data were examined by "ANOVA" to determine if statistical differences existed among the groups being tested. The statistical evaluations were performed on the Statistical Analysis System (SAS) in conjunction with the University of New Mexico Computer Science Center [14]. Further analysis was made by the Duncan's multiple range test to specifically determine which groups differed from each other and which groups were statistically equivalent. A p value of less than or equal to 0.05 was considered statistically significant.

RESULTS

The PRL Response to Acquisition Testing in Control and LM-Treated Rats

Figure 1 shows the PRL response of control and LM-treated rats to acquisition testing. Both groups showed statistically equivalent basal levels. On the first day of testing, control rats showed a significant 15-fold change in PRL levels in response to testing. LM infusions completely suppressed the increase in PRL levels that was seen on the first day of acquisition testing, and these levels remained at basal values throughout the experiment.

The Acquisition of a Conditioned Avoidance Response (CAR) in Control and LM-Treated Rats

Figure 2 shows the percent of CARs performed by the rats during the period of acquisition testing. With increasing periods of testing, the control rats showed steadily increasing CARs, reaching a level of some 70% after 7 days. This level of performance did not change significantly for the remaining two weeks of the experiment (data not shown). Rats receiving LM infusions did not show significantly different acquisition patterns.

DISCUSSION

In the present study we were unable to provide evidence that PRL acts to facilitate an adaptive conditioned response to a fearful stress situation. The idea that the hormonal stress response can, in some way, help an organism overcome the disruptive effect of a stressor has been a crucial link between "stress theory" and the concept of homeostasis [25]. However, as Mason [18] pointed out, how can the same hormonal response have "adaptive utility" in response to diverse stimuli? Mason answered this by saying that perhaps the only bodily response which might conceivably be appropriate, in the homeostatic sense, to diverse stimuli would be a behavioral response of emotional arousal preparatory to flight, struggle, or other strenuous exertion which might serve to eliminate the source of stress or remove the subject from the presence of the stressor. Thus, according to Mason, the "stress concept should not be regarded primarily as a physiological concept but rather as a behavioral concept." The present study has taken Mason's idea one step further and hypothesized that the PRL stress response may be able to aid in the removal of the subject from the presence of the stressor by facilitating the acquisition of an adaptive behavioral response. This hypothesis was tested by suppressing PRL secretion and then monitoring the animal's ability to acquire and perform an active avoidance response in a shuttle box paradigm.

While PRL levels have previously not been measured during avoidance conditioning, workers have generally shown that adrenal glucocorticoids rise during different types of avoidance testing [2, 11, 17]. Since avoidance conditioning is a highly stressful experience, it was not surprising that PRL levels also increased during early testing. However, we have recently found that PRL levels did not increase after one week of testing [27]. The corticosterone response has also been shown to decline after the CAR is acquired [2,6]. Thus, it would seem that if PRL and/or ACTH acted to enhance avoidance conditioning, it would be of significance only during the early testing period. In the present study, LM infusion completely blocked the increase of PRL seen during early test trials and maintained a low level of PRL for the duration of the test period. The rats treated with LM showed no difference in the acquisition performance of a CAR when compared to control rats. There was a possibility that LM treatment might produce changes in locomotor activity that would mask real differences in acquisition. Being aware of this, open-field observations were performed in both groups of rats after one week of acquisition testing. No significant differences in locomotor, rearing or grooming activity were found between the two groups (unpublished observation). It is, therefore, unlikely that performance differences, particularly in locomotion could explain the results.

Our results are not supportive of the hypothesis that the PRL increase seen in response to acquisition testing might be playing a role in facilitating acquisition performance. However, acquisition of an avoidance behavior as studied in the shuttle box and pole-jumping situations, was shown by Drago *et al.* [10] to be enhanced in hyperprolactinemic rats. These workers suggested that PRL may facilitate brain processes involved in avoidance acquisition. In support of that suggestion was the finding that congenitally PRL-deficient mice of the Dwarf strain have a reduced level of passive avoidance retention performance [3]. While the results of the present study do not support the finding by Drago *et al.* [10], it is worth remembering that PRL can alter dopamine activity in the CNS [1, 12, 24] and the dopamine may be involved in active avoidance behavior [4,5]. Therefore, it is possible that PRL could act to stimulate acquisition via a dopamine mechanism. If this were true, then it could help explain our negative finding. The LM, being a dopamine agonist, could be acting to promote acquisition, thus substituting functionally for the suppressed PRL.

REFERENCES

1. Annunziato, L. and K. E. Moore. Prolactin in CSF selectively increased dopamine turnover in the median eminence. *Life Sci* **22:** 2037–2042, 1978.
2. Berger, D. F., J. J. Starzec and E. B. Mason. The relationship between plasma corticosterone levels and leverpress avoidance vs. escape behaviors in rats. *Physiol Psychol* **9:** 81–86, 1981.
3. Bouchon, R. and B. Will. Learning performance in Dwarf mice. *Behav Brain Res* **2:** 247–248, 1981.
4. Cooper, B. R., G. R. Breese, L. D. Grant and J. L. Howard. Effect of 6-hydroxydopamine treatments on active avoidance responding: evidence for involvement of brain dopamine. *J Pharmacol Exp Ther* **185:** 358–370, 1973.
5. Cooper, B. R., J. L. Howard, L. D. Grant, R. D. Smith and G. R. Breese. Alteration of avoidance and ingestive behavior after destruction of central catecholamine pathways with 6-hydroxydopamine. *Pharmacol Biochem Behav* **2:** 639–649, 1974.
6. Coover, G. D., H. Ursin and S. Levine. Plasma corticosterone levels during active-avoidance learning in rats. *J Comp Physiol Psychol* **82:** 170–174, 1973.
7. de Wied, D. Effect of peptide hormones on behavior. In: *Frontiers of Neuroendocrinology*, edited by W. F. Ganong and L. Martini. New York: Oxford University, 1969, pp. 97–140.
8. Drago, F., P. L. Canonico, R. Bitetti and U. Scapagnini. Systemic and intraventricular prolactin induces excessive grooming. *Eur J Pharmacol* **65:** 457–458, 1980.
9. Drago, F., J. M. Van Ree, B. Bohus and D. de Wied. Endogenous hyperprolactinaemia enhances amphetamine and apomorphine-induced stereotypy. *Eur J Pharmacol* **72:** 249–253, 1981.
10. Drago, F., B. Bohus and J. A. M. Mattheij. Endogenous hyperprolactinemia and avoidance behaviors of the rat. *Physiol Behav* **28:** 1–4, 1982.
11. Feldman, J. and G. M. Brown. Endocrine responses to electric shock and avoidance conditioning in the rhesus monkey: cortisol and growth hormone. *Psychoneuroendocrinology* **1:** 231–242, 1976.
12. Gudelsky, G. A., J. Simpkins, J. Meites and K. E. Moore. Effects of prolactin and haloperidol on dopamine turnover in the tubero-infundibular and nigrostriatal pathways in the rat brain. *Soc Neurosci Abstr* **2:** 671, 1976.
13. Hansen, B. L., G. N. Hansen and C. Hagen. Immunoreactive material resembling ovine prolactin in perikarya and nerve terminals of the rat hypothalamus. *Cell Tissue Res* **226:** 121–131, 1982.
14. Helwig, J. J. and K. A. Council (eds.) *SAS Users Guide*. Cary, NC: SAS Inst., Inc., 1979.
15. Konincky, P. Stress hyperprolactinemia in clinical practice. *Lancet* **1:** 273, 1978.
16. Krulich, L., E. Hefco, P. Illner and C. B. Read. The effect of acute stress on the secretion of LH, FSH, prolactin and GH in the normal male rat with comments on their statistical evaluation. *Neuroendocrinology* **16:** 293–311, 1974.
17. Mason, J. W. A review of psychoendocrine research on the pituitary-adrenal cortical system. *Psychosom Med* **30:** 576–607, 1968.
18. Mason, J. W. A re-evaluation of the concept of non-specificity in stress theory. *J Psychiatr Res* **8:** 323–333, 1971.
19. Mills, D. E., M. T. Buckman and G. T. Peake. A chronically hyproprolactinemic rat model: administration of lergotrile mesylate by osmotic minipump. *Proc Soc Exp Biol Med* **166:** 438–441, 1981.
20. Nicholson, G., G. H. Greeley, J. Humm, W. W. Youngblood and J. S. Kizer. Prolactin in cerebrospinal fluid: a probable site of prolactin autoregulation. *Brain Res* **190:** 447–457, 1980.
21. Nicoletti, F., R. M. DiGiorgio, F. Patt, L. Rampello and U. Scapagnini. Influence of hyperprolactinemia induced by adenopituitary transplantation under the kidney capsule on the glutamic acid decarboxylase activity in various brain regions. *Arch Int Pharmacodyn Ther* **249:** 153–157, 1981.
22. Nicoll, C. A., P. K. Talwalker and J. Meites. Initiation of lactation in rats by nonspecific stresses. *Am J Physiol* **198:** 1103–1106, 1960.
23. Noel, G. L., H. K. Suh and A. G. Frantz. Stimulation of prolactin release by stress in humans. *Clin Res* **19:** 718, 1971.
24. Perkins, N. A. and T. C. Westfall. The effect of prolactin on dopamine release from rat striatum and medial basal hypothalamus. *Neuroscience* **3:** 59–63, 1978.
25. Selye, H. *Hormones and Resistance*. New York: Springer-Verlag, 1971.
26. Toubeau, G., J. Desclin, M. Parmentier and J. L. Pasteels. Compared localizations of prolactin-like and adrenocorticotropin immunoreactivities within the brain of the rat. *Neuroendocrinology* **29:** 374–384, 1979.
27. Yelvington, D. B., G. K. Weiss and A. Ratner. Plasma prolactin levels during conditioned avoidance behavior in rats. *Physiol Behav* **34:** 441–444, 1985.