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Vasopressin Stimulation of Acetate Incorporation into Lipids in a Dimethylbenz(*a*)anthracene-induced Rat Mammary Tumor Cell Line[1]

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Abstract

In a preliminary report we described the effects of rat prolactin on the incorporation of [^{14}C]acetate into lipids by a cell line derived from a dimethylbenz(*a*)anthracene-induced rat mammary tumor. The characteristics of the response to prolactin were very similar to those described for the normal rat mammary gland; namely, insulin was required for full expression of the response, maximal activity was not seen until 36 hr after the addition of the hormones, and growth hormone was able to elicit the same response. However, we were unable to detect binding of ^{125}I-labeled prolactin to these cells, and furthermore, other more purified prolactin preparations were inactive. Upon further investigation we discovered that the activity resided in a low-molecular-weight fraction of the rat prolactin B-1 preparation and was probably either vasopressin or oxytocin or both. These data suggest the possibility that vasopressin may play a role in rodent mammary tumorigenesis.

Introduction

Recently, some of the activity originally attributed to "prolactin" has been found to be due to vasopressin contamination (1, 3, 6). This observation has been limited to alleged effects of prolactin on salt and water metabolism. We would like to report here a similar observation with respect to an effect of prolactin on acetate incorporation into lipids in cells derived from a rat mammary tumor (8).

Materials and Methods

Materials. Radioactive sodium [2-^{14}C]acetate (59 mCi/mmol) was purchased from Amersham/Searle (Arlington Heights, Ill.). Rat prolactin (NIH preparation B-1, 7 IU/mg), rat prolactin (NIH preparation I-2, 30 IU/mg), and rat growth hormone (NIH preparation B-3, 0.9 IU/mg) were supplied by A. F. Parlow of the National Institute of Arthritis, Metabolism, and Digestive Diseases Rat Pituitary Hormone Distribution Program. Human growth hormone (NIH preparation HS-2019G, 1.8 IU/mg) and ovine prolactin (NIH preparation P-S-12, 35 IU/mg) were a gift of the NIH Pituitary Hormone Distribution Program. Porcine insulin (<0.002% glucagon) was the gift of Dr. Mary Root of Eli Lilly and Co. (Indianapolis, Ind.). Dexamethasone was purchased from Steraloids, Inc. (Wilgon, N. H.). Vasopressin and oxytocin were obtained from Calbiochem (San Diego, Calif.). Both peptides were synthetic and chromatographically pure. Vasopressin was free of oxytocin activity, and oxytocin was free of vasopressin activity.

Cell Cultures. WRK-1 is a cloned cell line derived from a dimethylbenz(*a*)anthracene-induced rat mammary tumor. A detailed description of culture techniques and cell morphology will be reported elsewhere in these proceedings. In brief, the cells have been maintained in continuous culture for more than 100 passages since cloning. They have a modal chromosome number of 80. Growth in soft agar is marginal, and transplantation into s.c. fat or under the kidney capsule fails to produce tumors in an isologous host. There are no α-lactalbumin by enzyme assay and no casein messenger RNA by hybridization to a complementary DNA probe.

The cells were grown in Eagle's minimal essential medium supplemented with L-glutamine (0.6 g/liter), penicillin, and streptomycin minimal essential medium plus 10% fetal calf serum (North American Biologicals, Inc., Miami, Fla.) and 5% rat serum (Grand Island Biological Co., Grand Island, N. Y.) at 37° in a humidified incubator in a 5% CO_2-95% air atmosphere. The stock cells were harvested with 0.05% trypsin-0.02% EDTA solution and replicately plated into 6-well (35-mm) plastic dishes (Linbro Chemical Co., New Haven, Conn.). When the cells were confluent, the medium was changed to minimal essential medium without serum. Six hr later, the medium was changed again and hormones were added.

Measurement of Acetate Incorporation. The incorporation of [^{14}C]acetate into fatty acids was measured as previously described (9). Incorporation was linear with time for up to 4 hr.

Filtration of Rat Prolactin (B-1). Rat prolactin (0.5 mg/ml) was dissolved in Dulbecco's phosphate-buffered saline (pH 7.4) without calcium or magnesium and filtered under pressure through an Amicon PM-10 filter, which has a 10,000-molecular weight cutoff. The residue remaining was washed several times, and both the filtrate and residue were assayed for activity. When ^{125}I-labeled prolactin was filtered, less than 3% of the radioactivity passed through the filter.

Results

Effect of Hormones on Incorporation of [^{14}C]Acetate into Lipids. Chart 1 shows the effect of hormones on the incorporation of [^{14}C]acetate into lipids by WRK-1 cells. Rat prolactin B-1 has a minimal effect by itself; however, in the presence of insulin there are a 3-fold increase in incorporation above levels seen with insulin alone and an overall 20-fold stimulation above controls. The synthetic glucocorticoid dexamethasone has a slight inhibitory effect in this experiment; however, this inhibition is not always observed

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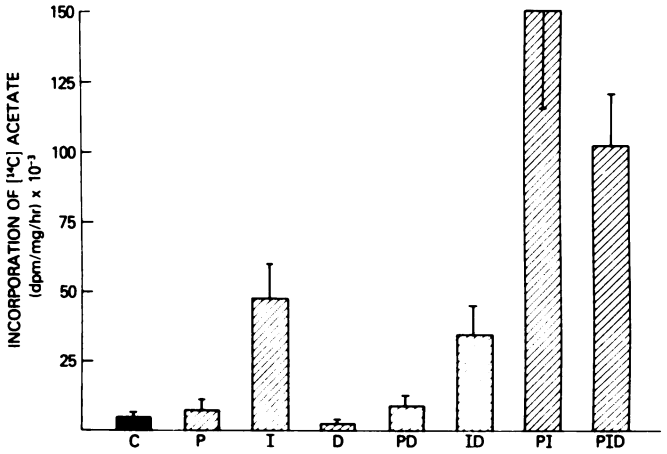

Chart 1. Effect of hormones on the incorporation of [^{14}C]acetate into lipids by WRK-1 cells. Following 36 hr of hormone treatment, cells were pulsed for 2 hr with [^{14}C]acetate, 1 μCi/ml. *C*, control; *P*, rat prolactin B-1 preparation (1 μg/ml); *I*, insulin (5 × 10^{-7} M); *D*, dexamethasone (10^{-7} M). Values represent the mean of triplicate determinations ± S.D.

and appears to be a function of the density of the cells (data not shown).

Prolactin Dose-Response Curves. The effects of prolactin B-1 alone and in the presence of insulin are presented in Chart 2. There is an approximately 2-fold increase in incorporation of [^{14}C]acetate with prolactin B-1 alone. In the presence of insulin, the effect is increased to 10-fold. Concentrations of prolactin B-1 required for maximal stimulation in both instances are in the range of 1 to 5 μg/ml. Results seen with prolactin alone are generally less consistent than those obtained when insulin is present. However, in both cases the fold induction varies. At least one determinant of the level of induction is the density of the cells, with greater stimulations apparent at lower densities.

Effect of "Growth Hormone". Because growth hormone preparations have been shown to stimulate fat synthesis in rodent mammary glands (4), we tested the ability of rat growth hormone B-3 to stimulate acetate incorporation in the presence of insulin. When dose-response curves for growth hormone B-3 and prolactin B-1 were compared, they were found to be similar (Chart 3). Ovine and bovine growth hormone were also active (data not shown).

Effect of Other Lactogenic Hormone Preparations. Because the dose-response curves for prolactin (B-1) and growth hormone (B-3) were so similar and because relatively high concentrations of each were required for response, we wondered whether a common contaminant was responsible for the observed response. We therefore tested other lactogenic hormone preparations (Chart 4). Rat prolactin B-1 exhibited its usual activity. However, more purified rat prolactin I-2, human growth hormone HS-2019G, and ovine prolactin P-S-12 had minimal effects on the incorporation of [^{14}C]acetate into lipids. Activity appeared to be inversely related to the purity of the preparation. Rat prolactin B-1 has a specific activity of 7 IU/mg, while the I-2 preparation and the ovine prolactin P-S-12 preparation have specific activities in the range of 30 IU/mg. The human growth hormone has a specific activity of 1.8 IU/mg, while the rat growth hormone has only 0.9 IU/mg. These data provide strong support for the contaminant hypothesis.

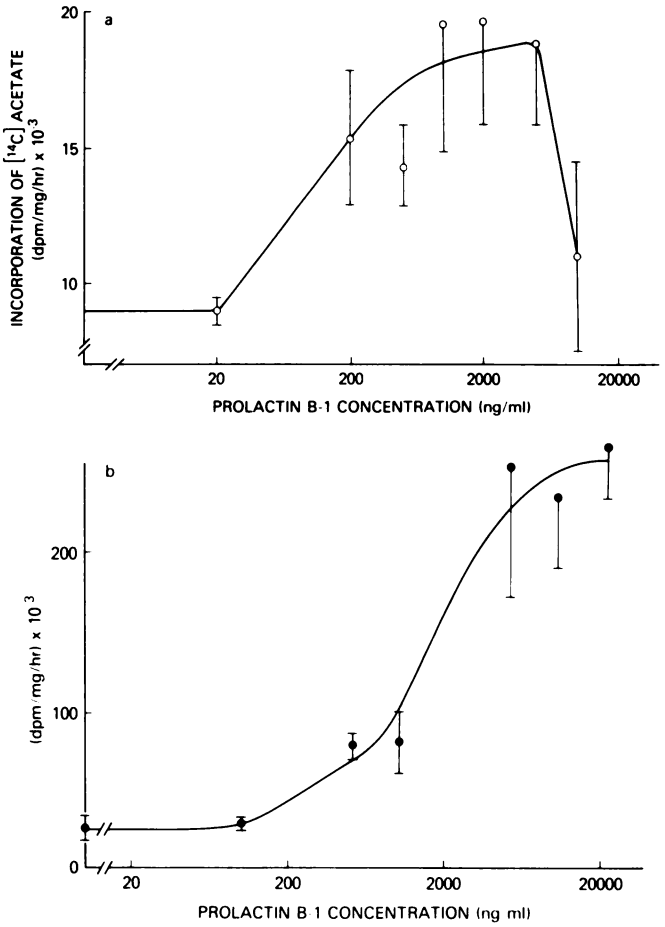

Chart 2. Effect of increasing concentrations of rat prolactin B-1 preparation on the incorporation of [^{14}C]acetate into lipids by WRK-1 cells. *a*, B-1 alone; *b*, B-1 plus 5 × 10^{-7} M insulin. Methods are as described for Chart 1. Values represent the mean of triplicate determinations ± S.D.

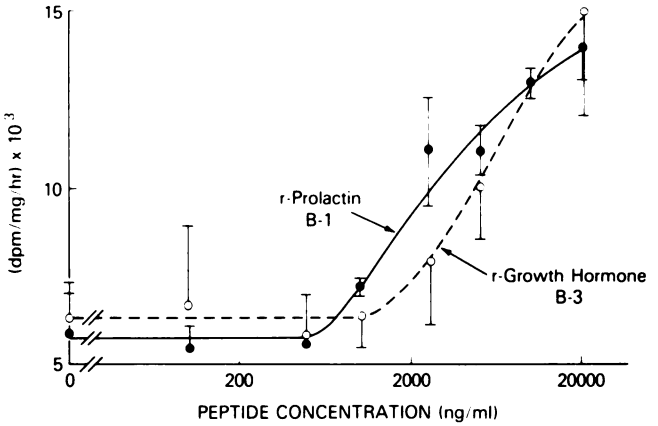

Chart 3. Effect of rat growth hormone B-3 preparation on the incorporation of [^{14}C]acetate into lipids by WRK-1 cells. Each dish contained 10^{-6} M insulin. Methods are as described in Chart 1. ●, rat prolactin B-1; ○, rat growth hormone B-3. Values represent the mean of triplicate determinations ± S.D.

Effect of Vasopressin and Oxytocin. Table 1 illustrates that both vasopressin and oxytocin are capable of eliciting the response observed with impure prolactin and growth hormone preparations. Concentrations of vasopressin in the range of 10^{-10} M (20 microunits/ml) are active. Approxi-

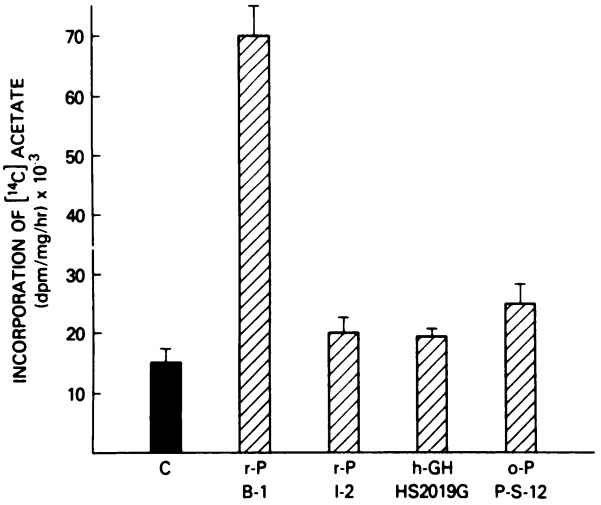

Chart 4. Effect of various lactogenic hormone preparations on the incorporation of [^{14}C]acetate into lipids by WRK-1 cells. Each dish contained 10^{-6} M insulin. Methods are as described in Chart 1. Abbreviations and hormone concentrations as follows: *C*, control; *r-P (B-1)*, rat prolactin B-1 (7 IU/mg, 2 μg/ml); *r-P (I-2)*, rat prolactin I-2 (30 IU/mg, 2 μg/ml); *h-GH (HS2019G)*, human growth hormone (1.8 IU/mg, 2 μg/ml); *o-P* (P-S-12), ovine prolactin P-S-12 (35 IU/mg, 2 μg/ml. Values represent the mean of triplicate determinations ± S.D.

Table 1

Effects of vasopressin and oxytocin on incorporation of [^{14}C]acetate into lipids by WRK-1 cells

[Vasopressin][a] (M)	Insulin (10^{-6} M)	Incorporation (dpm/mg/hr)	Fold stimulation
0	–	5,196 ± 632	
8×10^{-11}	–	11,231 ± 964	2.2
8×10^{-10}	–	18,105 ± 1,577	3.5
8×10^{-9}	–	20,979 ± 1,003	4.0
0	+	34,229 ± 7,208	
8×10^{-11}	+	62,666 ± 1,708	1.8
8×10^{-10}	+	123,111 ± 5,153	3.6
8×10^{-9}	+	144,845 ± 13,473	4.2
[Oxytocin][a] (M)			
0	–	8,252 ± 1,277	
4×10^{-7}	–	9,373 ± 399	1.1
4×10^{-8}	–	24,716 ± 7,010	3.0
4×10^{-7}	–	29,056 ± 6,035	3.5
0	+	34,229 ± 7,208	
4×10^{-9}	+	48,619 ± 2,617	1.4
4×10^{-8}	+	72,405 ± 9,493	2.1
4×10^{-7}	+	111,625 ± 3,548	3.3

[a] Concentrations are calculated on the basis of the following specific activities of the pure peptides. Lysine vasopressin, 250 IU/mg; oxytocin, 500 IU/mg (2).

mately 100-fold more oxytocin is required, suggesting that vasopressin is the physiologically relevant hormone.

Filtration of Rat Prolactin (B-1). To confirm the hypothesis that the activity present in rat prolactin B-1 was due to a low molecular weight contaminant, the prolactin was filtered through an Amicon PM-10 filter, and both filtrate and residue assayed for activity. Results are seen in Chart 5. Virtually all the activity resides in the low-molecular-weight filtrate, although in this experiment some activity is seen in the high-molecular-weight fraction as well. However, in other experiments, this fraction is totally devoid of stimulatory activity.

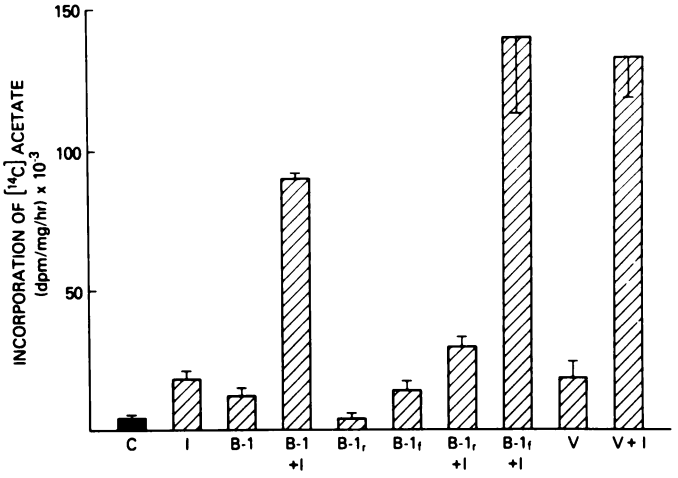

Chart 5. Amicon PM-10 filtration of rat prolactin B-1 preparation. Methods are as described in "Materials and Methods." *C*, control; *I*, insulin (10^{-6} M); *B-1*, rat prolactin B-1 (5 μg/ml); *B-1$_f$*, that fraction that did not pass through the PM-10 filter; *B-1$_f$*, that fraction that passed through the PM-10 filter; *V*, vasopressin (8×10^{-10} M). Values represent the mean of triplicate determinations ± S.D.

Discussion

Effects of prolactin on a variety of lactogenic functions as well as on salt and water metabolism have been described in (5, 10, 11). It has been suggested that the salt and water activity of prolactin is due to vasopressin contamination (3, 6). This raises the possibility that some of the reported effects of prolactin preparations on the mammary gland may be due to vasopressin as well. High concentrations of prolactin (1 to 5 μg/ml) are routinely used to stimulate mammary gland explants (4, 11). At these concentrations significant amounts of other contaminating hormones may be present. NIH ovine prolactin P-S-10, for example, has been reported to contain 20 ng of vasopressin per mg of prolactin (6); thus, 5 μg/m of prolactin would result in a vasopressin concentration of 100 pg/ml or approximately 45 microunits/ml, which is supraphysiological [physiological concentrations range from 4 to 40 pg/ml (7)].

We have demonstrated that the response of a rat mammary tumor cell line to rat prolactin and growth hormone was actually due to a contaminant of lower molecular weight. Both purified vasopressin and oxytocin are active in this system. The observed activity would require a minimal contamination with vasopressin of approximately 0.02% and with oxytocin of approximately 2%. When the hormone preparations used in this study were assayed for vasopressin by radioimmunoassay, the levels of vasopressin observed closely correlated with the activity of a given preparation. Active preparations, such as rat prolactin (B-1) and rat growth hormone (B-3), for example, contain 438 (0.04%) and 332 (0.03%) ng of vasopressin per mg of protein, respectively, while human growth hormone, which is inactive, contains only 6 ng/mg. Thus, vasopressin is probably responsible for the activity observed with rat prolactin and growth hormone.

The results reported here have an important implication; namely, some of the tumorigenic and/or lactogenic properties attributed to prolactin and growth hormone may rather reflect an action of vasopressin. We are currently investigating this possibility. In the future, investigators

should be careful in documenting that hormone preparations are free of other potentially active contaminants.

Acknowledgments

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