Effect of Hyperthermically Elevated Serum Prolactin on Epididymal Cyclic AMP (Adenosine 3′,5′-Cyclic Monophosphate) Levels in Rats

J. M. ROSENBERG ×, I. S. KAMPA *, V. REID, J. M. HUNDERTMARK *, D. W. FRASCELLA ‡, and C. A. LAU-CAM §

Received June 21, 1979, from the *Arnold and Marie Schwartz College of Pharmacy and Health Sciences, Brooklyn, NY 11201.* Accepted for publication October 5, 1979. *Department of Pathology, Valley Hospital, Ridgewood, NJ 07451. ‡Hoechst-Roussel Pharmaceuticals, Somerville, NJ 08876. §Department of Pharmaceutical Sciences, St. John's University College of Pharmacy and Allied Health Professions, Jamaica, NY 11439.

Abstract □ To investigate whether increased blood prolactin concentration affects epididymal cyclic AMP (adenosine 3′,5′-cyclic monophosphate) levels during hyperthermic stress, a study was conducted on 40 Sprague–Dawley male rats divided into four equal groups. Two groups were injected intraperitoneally with bromocriptine, a potent inhibitor of prolactin release. Under identical experimental conditions, one group was subjected to hyperthermic stress, and the other group was maintained at room temperature. Two additional groups were injected with a control vehicle; one group was subjected to hyperthermic stress, and the other group was kept at normal body temperature. Blood samples were analyzed for serum prolactin, and epididymal fat pads were assayed for cyclic AMP. Serum prolactin levels increased significantly ($p < 0.01$) in hyperthermic rats, but this effect was suppressed completely by bromocriptine. Cyclic AMP levels were increased ($p < 0.01$) during elevated body temperature in all animals examined, with no significant difference ($p > 0.05$) occurring between the nonbromocriptine-treated and bromocriptine–prolactin-suppressed groups. This investigation suggested that elevation of epididymal fat cyclic AMP during hyperthermia is not the result of increased prolactin levels.

Keyphrases □ Cyclic AMP—epididymal levels, effect of hyperthermically elevated serum prolactin, rats □ Hyperthermia—elevation of serum prolactin, effect on epididymal cyclic AMP levels, rats □ Prolactin, serum—hyperthermic elevation, effect on epididymal cyclic AMP levels, rats

Hyperthermic stress induces changes in lipid metabolism, including a rise in epididymal fat pad cyclic AMP (adenosine 3′,5′-cyclic monophosphate) (1–3). It has been shown that insulin exerts an inhibiting effect on the rise of epididymal fat pad cyclic AMP during hyperthermia (1). However, the rise in cyclic AMP may result from a concomitant decrease in blood insulin (1), an increase in extrapituitary lipolytic hormones such as glucagon (1), and/or an increase in pituitary lipolytic hormones such as prolactin (4–6). The present study was designed to elucidate the role of prolactin on the elevation of epididymal cyclic AMP during hyperthermic stress.

EXPERIMENTAL

Animals—Forty male Sprague–Dawley adult rats[1], 225–300 g, were divided into four equal groups. They were housed for 21 days prior to the study in a temperature-controlled (23 ± 3°), artificially illuminated (lights on from 7:00 am to 7:00 pm daily) room. During this period, the rats were given food[2] and water *ad libitum*. Prior to all experiments, the animals were fasted for 12 hr.

Effect of Hyperthermia on Epididymal Fat Pad Cyclic AMP and Serum Prolactin—The first 10 animals (hyperthermic bromocriptine group) were injected intraperitoneally with 1 mg of bromocriptine[3], a known potent inhibitor of prolactin (7), in 0.5 ml of 5% ethanol. A second group of animals (hyperthermic control group) was injected intraperitoneally with 5% ethanol vehicle. Thirty minutes after injection, both groups were placed in an environmental chamber[4]. The relative humidity was maintained between 40 and 45% within the chamber, and the ambient temperature was controlled to allow the rectal temperature[5] to increase ~1°/30 min until a value of 42 ± 0.1° was reached. This rectal temperature was maintained for approximately 30 min, at which time blood samples were obtained by decapitation. A small portion of epididymal fat was removed immediately from each animal and frozen in liquid nitrogen.

The third group of animals (normothermic bromocriptine group) was treated as described for the first group, and the remaining 10 animals (normothermic control group) were treated identically to the second group. For the latter two groups, the temperature in the environmental chamber was maintained at 23 ± 3° for the 3 hr that the animals were contained in it. Blood and epididymal fat samples then were obtained.

Sample Collection and Assay—All decapitations were conducted between 3:30 and 5:00 pm. Blood was collected from the trunk portion and allowed to stand for 10 min at 4°, and the serum was separated and frozen at −40° until hormone analysis could be performed. Serum prolactin levels were measured using a double antibody radioimmunoassay kit[6] according to the instructions supplied. The rat prolactin was iodinated by a modification of the Hunter–Greenwood method (8). Each serum sample was assayed in duplicate, and the average was taken as representative of the true prolactin concentration. All results are expressed in terms of nanograms per milliliter of NIAMDD rat prolactin.

Cyclic AMP was extracted from the epididymal fat pads by a method described by Steiner *et al.* (9), and the concentrations were determined by radioimmunoassay[7] using a modification of a method reported by the same investigators (10). Each sample was assayed in duplicate, and the average was taken as representative of the concentration. Cyclic AMP results are expressed in picomoles per gram of wet epididymal fat tissue.

Statistical comparisons were determined by the Student t test (11).

RESULTS

Figure 1 shows the results of hyperthermic stress on serum prolactin levels following treatment with bromocriptine or control vehicle. Prolactin levels were elevated significantly ($p < 0.01$) in the hyperthermic control group compared to the normothermic control group [124.3 ± 20.6 (*SE*) *versus* 44.8 ± 7.7 (*SE*) ng/ml, respectively; $t = 3.4$, $df = 18$]. Prolactin levels were depressed significantly ($p < 0.01$) in the hyperthermic bromocriptine group compared to the hyperthermic control group [11.2 ± 1.2 (*SE*) *versus* 124.3 ± 20.6 (*SE*) ng/ml, respectively; $t = 39.0$, $df = 18$] and in the normothermic bromocriptine group compared to the normothermic control group [44.8 ± 7.7 (*SE*) *versus* 13.4 ± 1.2 (*SE*) ng/ml, respectively; $t = 3.5$, $df = 18$], with no significant difference ($p > 0.05$) between the bromocriptine–prolactin-suppressed groups [11.2 ± 1.2 (*SE*) *versus* 13.4 ■ 1.2 (*SE*) ng/ml, respectively; $t = 1.2$, $df = 18$].

Figure 2 indicates that epididymal cyclic AMP levels were elevated significantly ($p < 0.01$) in the hyperthermic control group compared to

[1] Taconic Farms, Germantown, N.Y.
[2] Purina Lab Chow, Ralston Purina Co., St. Louis, Mo.
[3] Sandoz Laboratories, East Hanover, N.J.
[4] Refrigerator incubator model 305500, Hotpack Corp., Philadelphia, Pa.
[5] Rectal thermister probe, Yellow Springs Instrument Co., Springfield, Ohio.
[6] Supplied by the National Institutes of Arthritis, Metabolism, and Digestive Diseases (NIAMDD) Rat Pituitary Hormone Distribution Program, National Pituitary Agency, Baltimore, Md.
[7] Cyclic AMP radioimmunoassay kit ^{125}I, Becton Dickinson, Orangeburg, N.Y.

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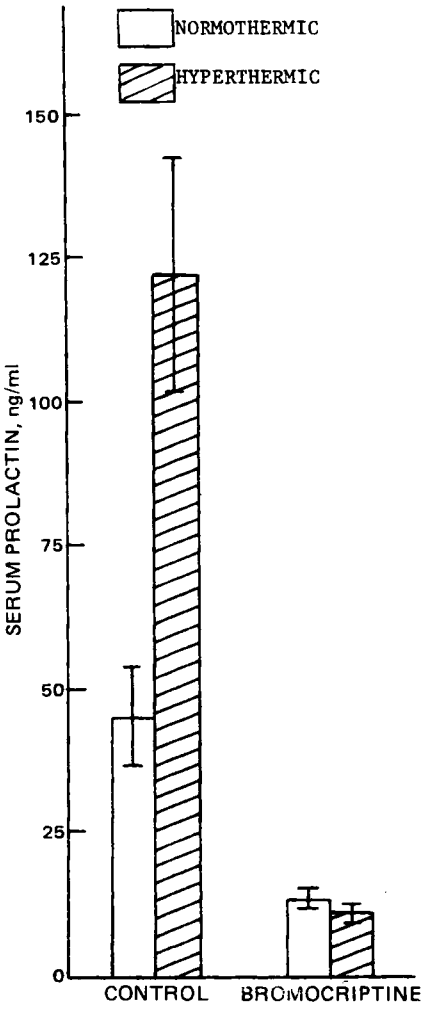

Figure 1—*Serum prolactin levels in rats treated with bromocriptine or vehicle during normal and elevated rectal temperatures. Each vertical bar represents the mean of 10 observations. The vertical brackets indicate standard errors of the mean. Prolactin levels in the hyperthermic control rats were significantly ($p < 0.01$) elevated compared to normothermic controls. Bromocriptine treatment significantly depressed serum prolactin levels in both hyperthermic and normothermic animals.*

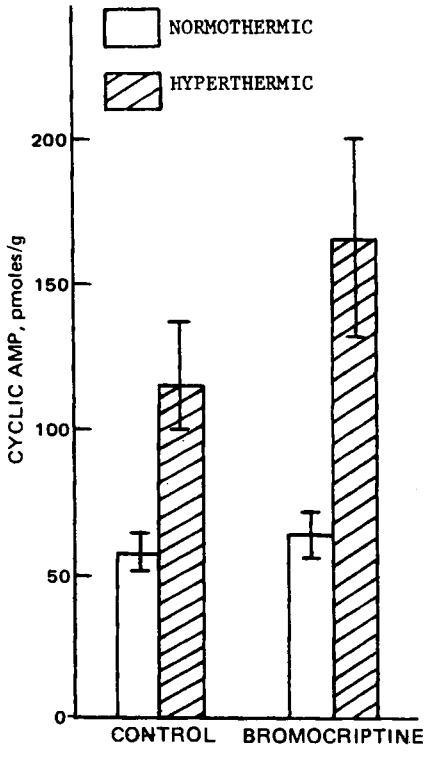

Figure 2—*Cyclic AMP concentrations in rats treated with bromocriptine or control vehicle during normal and elevated rectal temperatures. Each vertical bar represents the mean of 10 observations. The vertical brackets indicate standard errors of the mean. Hyperthermia significantly ($p < 0.01$) elevated cyclic AMP in both bromocriptine- and control vehicle-treated animals with no significant difference ($p > 0.05$) in the concentration of both groups.*

the normothermic control group [115.2 ± 20.9 (*SE*) *versus* 56.1 ± 6.7 (*SE*) pmoles/g, respectively; $t = 3.8$, $df = 18$] and in the hyperthermic bromocriptine group compared to the normothermic bromocriptine animals [165.7 ± 33.8 (*SE*) *versus* 63.4 ± 8.3 (*SE*) pmoles/g, respectively; $t = 3.9$, $df = 18$]. The cyclic AMP concentrations of the normothermic control and the normothermic bromocriptine groups were not significantly different ($p > 0.05$) [56.1 ± 6.7 (*SE*) *versus* 63.4 ± 8.3 (*SE*) pmoles/g, respectively; $t = 2.0$, $df = 18$], and there also was no significant difference ($p > 0.05$) in concentration between the hyperthermic control and the hyperthermic bromocriptine groups [115.2 ± 20.9 (*SE*) *versus* 165.7 ± 33.8 (*SE*) pmoles/g, respectively; $t = 1.2$, $df = 18$].

DISCUSSION

Serum prolactin levels are largely controlled by a hypothalamic prolactin-inhibiting factor related to dopamine which, indeed, may be dopamine (12). Pharmacological agents that reduce serum prolactin levels include dopamine agonists such as bromocriptine (7). Although bromocriptine also may transiently increase the prolactin clearance from plasma by stimulating its uptake into peripheral receptor sites, its exact inhibitory mechanism is not fully known (13).

In the present study, serum prolactin levels significantly increased in hyperthermic rats. Bromocriptine-treated animals exhibited suppressed serum prolactin levels, and no elevation of prolactin levels occurred during hyperthermia (Fig. 1). Previous studies (14, 15) showed that increased temperature raises blood prolactin. However, these changes were not attributed simply to stress since lowered temperature significantly decreased serum prolactin and elevated temperature produced the opposite effect (14, 15). Although it was reported previously that prolactin increases lipolysis in adipose tissue from mature rats, as indicated by an increased free fatty acid and glycerol release (16, 17), this effect has not been observed in young rats (16).

Prolactin also is known to increase cyclic AMP in some tissues but not in others (16). Since no significant change in the levels of cyclic AMP occurred among the bromocriptine-treated hyperthermic animals in contrast to nonbromocriptine-treated rats (Fig. 2), it appears that epididymal fat cyclic AMP elevations during hyperthermia occur independently of increases in serum prolactin levels. The role of extrapituitary lipolytic hormones such as glucagon is now being investigated to determine if they play a part in the epididymal fat cyclic AMP increase observed during elevated body temperature.

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In Vitro Adsorption Studies of Cimetidine

F. GANJIAN, A. J. CUTIE, and T. JOCHSBERGER ˣ

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Abstract □ The adsorption of cimetidine on selected pharmaceuticals including kaolin, activated charcoal, talc, and nonsystemic antacids was determined at pH 5.0 and 25°. The Langmuir and Freundlich adsorption isotherms showed that cimetidine adsorption was significant with activated charcoal, kaolin, talc, and magnesium trisilicate and was virtually nonexistent with magnesium hydroxide and aluminum hydroxide. Equations expressing the Freundlich and Langmuir adsorption isotherms were evaluated for each adsorbent. The approximate amounts of cimetidine adsorbed per gram of adsorbent were 25.6, 0.402, 0.291, and 0.343 mg for charcoal, kaolin, talc, and magnesium trisilicate, respectively. These *in vitro* studies indicate that some cimetidine may be lost when it is administered concomitantly with pharmaceutical adsorbents.

Keyphrases □ Cimetidine—adsorption onto various pharmaceutical adsorbents, *in vitro* □ Adsorption—cimetidine, various pharmaceutical adsorbents, *in vitro* □ Interactions—adsorption of cimetidine onto various pharmaceutical adsorbents

Numerous pharmaceuticals contain substances that are capable of adsorbing various drugs and other compounds (1–8). These substances include clays such as kaolin and talc and silicates found in some antacid–antiflatulent preparations. The purpose of this study was to determine if any adsorption interaction occurred with some commonly employed pharmaceutical ingredients including kaolin, talc, magnesium trisilicate, aluminum hydroxide, magnesium hydroxide, activated charcoal, and the H_2-receptor antagonist cimetidine used for the treatment of duodenal ulcers and gastric hypersecretion. Since antacids are recommended commonly for concurrent therapy with cimetidine, it was of interest to determine if any *in vitro* interaction was evident. To evaluate the efficiency of the adsorption, activated charcoal was employed as a standard.

EXPERIMENTAL

Reagents—Activated charcoal[1], kaolin[1], talc[2], aluminum hydroxide[1], magnesium hydroxide[1], and magnesium trisilicate[3] were reagent grade. Cimetidine[4] was compendial grade and was used without further purification. Methanol was certified ACS spectroanalyzed grade[1].

Procedure—All potential adsorbents (aluminum hydroxide, charcoal, kaolin, magnesium hydroxide, magnesium trisilicate, and talc) employed were washed repeatedly with distilled water followed by spectroanalytical grade methanol until the wash solution exhibited no absorbance at 226 nm (the wavelength of maximum cimetidine absorption). The adsorbents were passed through a 20-mesh sieve[1] while still moist and permitted to dry in an oven at approximately 45°. The dried materials then were passed through a 100-mesh sieve to ensure that the particle sizes of all of the adsorbents were 100 mesh or smaller. One gram of adsorbent was used in all cases, except for charcoal where only 50 mg was utilized.

Adsorbate mixtures were prepared by diluting aqueous cimetidine solutions (~12 mg/liter) with appropriate amounts of distilled water. The final pH of the solutions was 5.0[1]. The solutions then were agitated vigorously[5] for 10 min at 25° and allowed to stand for 20 min. Twenty-five-milliliter aliquots of the supernate were drawn from each solution, centrifuged[6] for 20 min, and assayed spectrophotometrically[7] at 226 nm. The reference solutions were prepared in an identical manner as the sample, with the cimetidine solution replaced by distilled water.

RESULTS AND DISCUSSION

The results of the *in vitro* adsorption of cimetidine onto various substances are given in Tables I–III and Figs. 1 and 2. The data are presented as both Freundlich constants (Eq. 1) and Langmuir constants (Eq. 2) as well as in terms of the adsorption rate:

$$\frac{x}{m} = kC^n \qquad \text{(Eq. 1)}$$

$$\frac{x}{m} = \frac{\alpha C}{1 + \beta C} \qquad \text{(Eq. 2)}$$

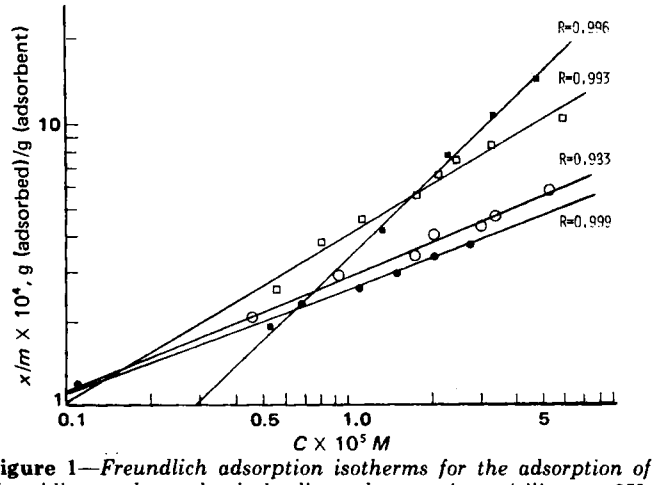

Figure 1—*Freundlich adsorption isotherms for the adsorption of cimetidine on charcoal, talc, kaolin, and magnesium trisilicate at 25°. Key:* ■, *magnesium trisilicate;* □, *kaolin;* ○, *talc; and* ●, *charcoal (values* × 10^2*).*

[1] Fisher Scientific Co., Fair Lawn, N.J.
[2] Matheson, Coleman and Bell, Norwood, Ohio.
[3] Mallinckrodt, St. Louis, Mo.
[4] Smith Kline and French Laboratories, Philadelphia, Pa.
[5] Shaker bath 6250, Eberbach Corp., Ann Arbor, Mich.
[6] Dynac centrifuge, Clay-Adams Co., New York, N.Y.
[7] Spectronic 200, Bausch & Lomb.

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