Does Dopamine Inhibit or Stimulate Prolactin Release *In Vitro*? The Effects of Dopamine Concentration and Duration of *In Vivo* Estradiol Treatment (43558)

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Abstract. **Prolactin release was examined from pituitary explants of ovariectomized, Fischer 344 rats at various times of estradiol treatment. The explants were acutely exposed to concentrations of dopamine from 0 to 10^{-4} *M* and the concentration of prolactin in the resulting incubation medium was determined by radioimmunoassay. At 1 and 2 weeks of estradiol treatment, prolactin release from explants of Fischer 344 rats was not inhibited by 10^{-6} or 10^{-5} *M* dopamine when compared with release from explants exposed to no dopamine, whereas dopamine at doses of 10^{-7} and 10^{-8} *M* significantly stimulated prolactin release. By 3 or 4 weeks of estradiol treatment, the stimulatory effects of the low doses of dopamine were not evident and higher doses significantly inhibited prolactin release. These data indicate that dopamine can inhibit, stimulate, or produce no change in prolactin secretion and that these effects depend upon the concentration of dopamine used and the duration of estradiol treatment to which the animals are subjected.** [P.S.E.B.M. 1993, Vol 202]

That dopamine potently inhibits rat prolactin secretion *in vitro* is well documented (see Ref. 1 for review), but not always true. Several laboratories have shown that dopamine, at very low doses, can stimulate prolactin release *in vitro* (2–6). In addition, the inhibitory action of dopamine on prolactin release is reduced by estradiol treatment (7–10). Our understanding of the actions of dopamine is complicated further by the observations that pituitary cells are heterogeneous with respect to their responsiveness to dopamine (6, 11) and the type of dopamine receptors they exhibit (12). In addition, the inhibitory effects of dopamine and the modulatory effects that estradiol has on the responsiveness of the pituitary to dopamine differ between strains of rats such that pituitary explants from Fischer 344 (F344) rats are resistant to the inhibitory action of 10^{-6} *M* dopamine early in estradiol treatment, whereas later in estradiol treatment this dose of dopamine effectively inhibits prolactin release (13). On the other hand, prolactin release from pituitary explants of Holtzman Sprague-Dawley rats is inhibited by dopamine early, but not later, in estradiol treatment (13). The objective of the present study was to extend these observations by determining dose-response relationships between dopamine and *in vitro* prolactin release from pituitaries of F344 rats treated with estradiol for varying periods of time.

Materials and Methods

Sexually mature female F344 rats were obtained from Harlan Laboratories (Indianapolis, IN) and housed in an environmentally controlled room (lights on, 0600–2000 hr; temperature, 23°C relative humidity, 40–50%). Food and tap water were available *ad libitum*. Two or three days after arrival, all rats were bilaterally ovariectomized under ketamine-xylazine anesthesia. Seven days later, all rats, except ovariectomized controls, were lightly anesthetized with ether or methoxyflurane and implanted subcutaneously with a 1.0-cm capsule made from Silastic tubing (no. 602-305: Dow-

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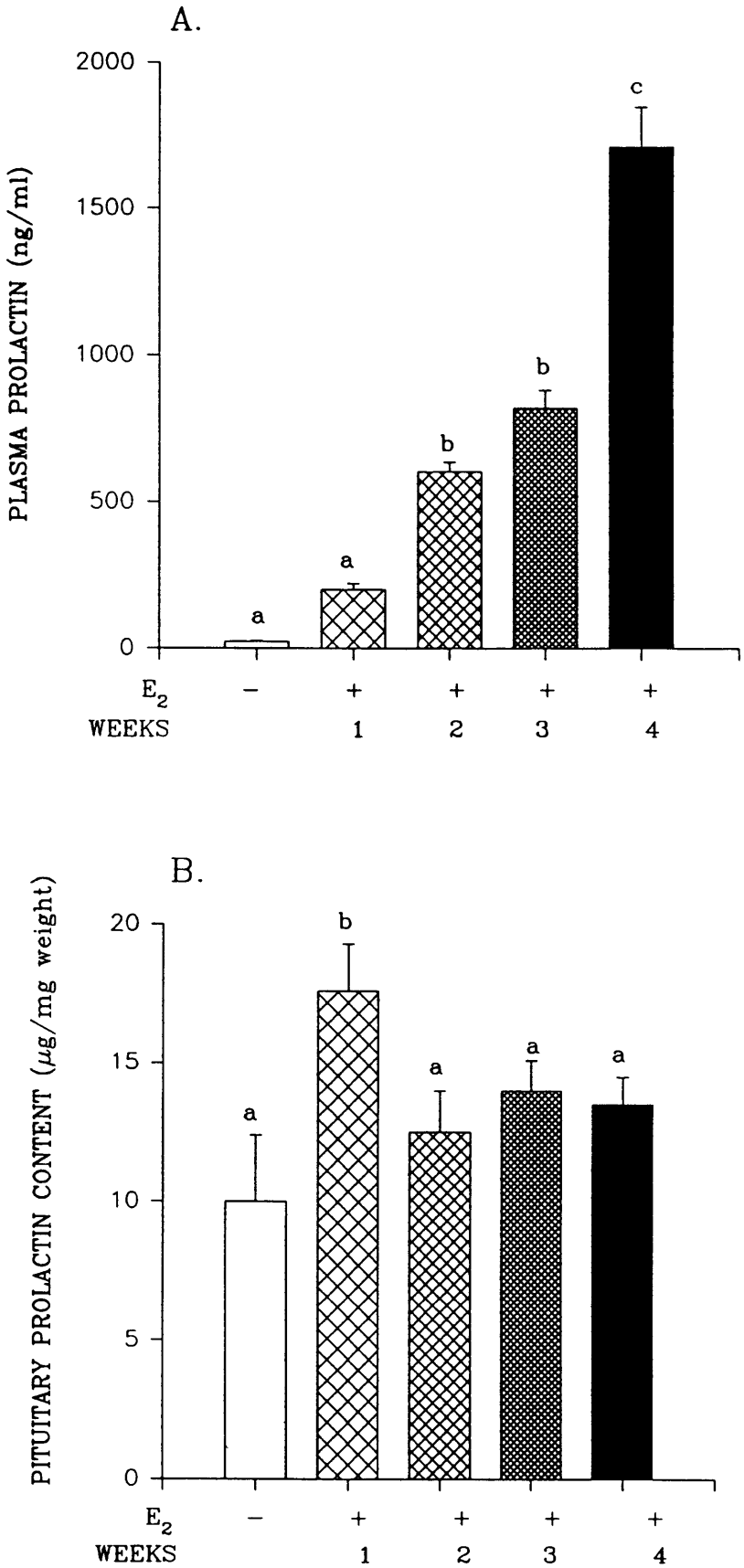
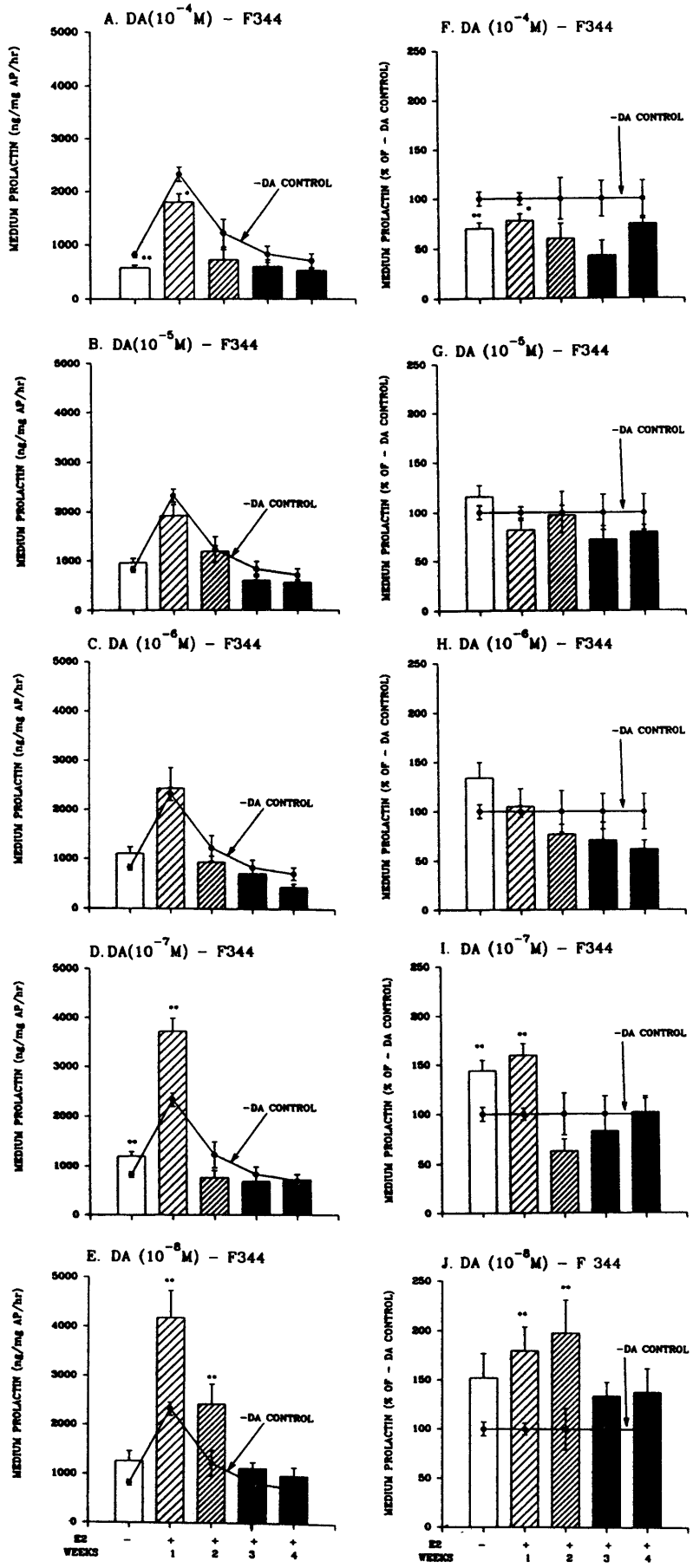


Figure 1. (A) Plasma levels and (B) pituitary content of prolactin in ovariectomized F344 rats treated with estradiol. Blood and anterior pituitaries were collected from groups of eight rats decapitated between 0900 and 1000 hr after a brief period (<1 min) of ether anesthesia. The anterior pituitaries were each cut into eight fragments and two of these were randomly selected, snap frozen on dry ice, and then weighed frozen and sonicated in phosphate-buffered saline, 0.1% bovine serum albumin, and 1% Triton X-100. Radioimmunoassay was used to measure prolactin in plasma and in pituitary sonicates. [a,b,c]Means with different symbols are statistically different ($P < 0.05$, Scheffe's test, $n = 8$).


A. DA(10^{-4}M) - F344
F. DA (10^{-4}M) - F344
B. DA(10^{-5}M) - F344
G. DA (10^{-5}M) - F344
C. DA (10^{-6}M) - F344
H. DA (10^{-6}M) - F344
D. DA(10^{-7}M) - F344
I. DA (10^{-7}M) - F344
E. DA (10^{-8}M) - F344
J. DA (10^{-8}M) - F 344
MEDIUM PROLACTIN (ng/mg AP/hr)
MEDIUM PROLACTIN (% OF - DA CONTROL)
-DA CONTROL
E2
WEEKS
- + + + +
1 2 3 4

Corning, Midland, MI) containing 10–15 mg of crystalline 17β-estradiol.

At 1, 2, 3, and 4 weeks of estradiol treatment, groups of eight rats were lightly anesthetized with ether and rapidly decapitated. A group of untreated, ovariectomized rats were also anesthetized and decapitated 7 days after ovariectomy as controls. Trunk blood was collected in heparinized tubes and the anterior pituitaries were rapidly removed. The blood was centrifuged and the plasma collected and frozen for prolactin radioimmunoassay. The anterior pituitaries were cut into eight fragments and two of these were rapidly frozen on dry ice to determine prolactin content. The remaining six explants were placed into wells of 24-well tissue culture plates containing 1 ml of α-modified minimal essential medium buffered with 20 m*M* $NaHCO_3$ and containing 0.1% bovine serum albumin, 0.1% ascorbic acid, and dopamine at concentrations of 10^{-4} to 10^{-8} *M* or no dopamine (as control). The explants were incubated in a humidified atmosphere of 95% O_2 and 5% CO_2 at 37°C for a 1-hr stabilization period, after which time they were transferred to wells containing fresh medium of identical composition to the first hour treatments; the incubation was continued for a second hour. Medium was recovered from the wells and stored at −20°C for prolactin assay. The nonincubated pituitary explants were weighed frozen and then sonicated for 30 sec in incubation medium containing 1% Triton X-100 using a Kontes micro-ultrasonic cell disrupter. These sonicates were stored at −20°C until assayed for prolactin.

Plasma, incubation medium, and pituitary sonicates were rapidly thawed, diluted with radioimmunoassay buffer (phosphate-buffered saline containing 0.1% bovine serum albumin), and assayed for prolactin in a double-antibody radioimmunoassay in two dilutions in duplicate according to the method of Kuo and Gala (14). The standard preparation for the RIA was NIDDK-RP-1 (11 IU/mg), the radiolabeled preparation was NIDDK-RP-I5 labeled with ^{125}I by the lactoperoxidase-glucose oxidase method of Tower *et al.* (15), and the primary antibody, which was made in rabbits against rat prolactin secreted *in vitro* and characterized as described previously (14), was provided by Dr. Richard Gala (Department of Physiology, Wayne State University).

Statistical comparisons of the means were done by one-way analysis of variance followed by post hoc comparisons using the Scheffe's test. Differences at the $P < 0.05$ level were considered statistically significant.

Results

Plasma levels of prolactin plotted against weeks of estradiol treatment are shown in Figure 1A. Prolonged estradiol treatment caused marked incremental increases in plasma prolactin over the 4 weeks of treatment such that by Week 4, levels were 1800 ng/ml. Pituitary content of prolactin is shown in Figure 1B. In contrast to the plasma levels pituitary prolactin content increased significantly at 1 week of estradiol treatment relative to ovariectomized controls, but then decreased to and remained at ovariectomized levels from 2 to 4 weeks of estradiol treatment.

Prolactin release from pituitary explants of F344 rats incubated in the presence of different concentrations of dopamine is shown in Figure 2 plotted against weeks of estradiol treatment. Figure 2, A through E, shows the absolute levels of prolactin released per milligram per hour, whereas Figure 2, F through J, shows the data as a percentage of the data obtained when dopamine was absent from the medium (−DA control). Prolactin released into medium in the presence or absence of dopamine was significantly higher at 1 week of estradiol treatment relative to the other treatment periods. This paralleled the temporal changes in pituitary prolactin content. Dopamine at 10^{-4} *M* significantly inhibited prolactin release from explants of ovariectomized control rats and rats treated for 1 week with estradiol, but not from explants obtained at 2, 3, or 4 weeks of estradiol treatment (Fig. 2, A and F). Dopamine at concentrations of 10^{-5} and 10^{-6} *M* did not inhibit prolactin release in any group (Fig. 2, B, C, G, and H), whereas concentrations of 10^{-7} and 10^{-8} *M* stimulated prolactin release by 50–100% from explants of ovariectomized rats and rats treated with estradiol for 1 or 2 weeks; however, at longer durations of estradiol treatment, these concentrations neither inhibited nor stimulated prolactin release (Fig. 2, D, E, I, and J).

Discussion

In the current study, dopamine inhibited, stimulated, or had no effect on prolactin release from pituitary explants *in vitro* depending on its concentration and the duration of *in vivo* estradiol treatment. These observations extend our previous findings, which showed that pituitaries from F344 rats are resistant to the inhibitory effects of dopamine early but not late in estradiol treatment (13).

That dopamine can stimulate prolactin release *in vitro* is not an original observation. Many laboratories have shown stimulatory effects (2–6). What is unique

Figure 2. Prolactin release into incubation medium from pituitary explants of ovariectomized or ovariectomized, estradiol-treated F344 rats in the presence or absence of dopamine. (A-E) Absolute prolactin release during 1-hr incubation period. (F-J) The same prolactin release data calculated as a percentage of the prolactin released in the absence of dopamine. The −DA Control data in Panels F through J were calculated as a percentage of the mean. $^*P < 0.05$; $^{**}P < 0.01$ + dopamine versus −dopamine within each treatment group (Scheffe's test, $n = 6$).

about the current findings is that dopamine stimulated prolactin release at doses that are several orders of magnitude higher than has previously been shown, and these higher doses are near the levels reported to be in the portal blood of ovariectomized, E_2-treated rats (10).

The current observations also indicate that the ultimate response to dopamine is strongly dependent upon the estrogen status of the animal. For example, dopamine stimulated prolactin release at 1 and 2 weeks of estrogen treatment, but not at 3 or 4 weeks. It is of interest that the stimulatory effects of dopamine were observed at a time when the pituitary explants were also resistant to the inhibitory effects of all but the highest concentration of dopamine. One possible interpretation is that the cellular mechanisms responsible for the stimulation may be normally masked by other more powerful, cellular responses that lead to inhibition of prolactin release and that estradiol effectively downregulates the inhibitory mechanisms allowing the stimulatory effects to be manifested. Another possibility is that different populations of lactotrophs may predominate in the pituitary at various times of estradiol treatment, with some populations being either sensitive or resistant to the inhibitory effects of dopamine whereas others may only be stimulated by dopamine. This interpretation is supported by recent reports (6, 12). Additional experiments are needed to examine the functional heterogeneity of lactotrophs in several strains of rats and in different physiologic states, but it can be concluded from the current observations that three signals exist with regard to the regulation by dopamine of prolactin secretion in rats, i.e., inhibition, loss of inhibition, and stimulation.

The interpretation of these *in vitro* studies should be tempered by the possibility that the *in vitro* responses were affected and perhaps confounded by the *in vivo* conditions to which the pituitary cells were exposed prior to their removal for study. In the current experiments, plasma levels of prolactin and prolactin content of the pituitary (Fig. 1), which reflect *in vivo* conditions at the time of sacrifice, were altered over the course of estradiol treatment, and the mechanisms responsible for these changes (e.g., portal blood concentrations of dopamine or estrogen-induced modifications in the lactotrophs) may have contributed to the differences observed in the *in vitro* release of prolactin. For example, pituitary stores of prolactin may have contributed to the ultimate responses seen. The pituitary content of prolactin was increased significantly at 1 week of E_2 treatment compared with ovariectomized controls. This was the time when significantly more prolactin was released *in vitro* and, coincidently, it was also the time when dopamine produced the greatest stimulation of prolactin release. Pituitary stores of prolactin decreased later in estrogen treatment as was also shown by us previously (16), probably due to increased release *in vivo* as indicated by the increased level of prolactin in the plasma, and the stimulatory effects of dopamine disappeared. Although pituitary concentration of prolactin may have influenced the ultimate response to dopamine, it seems more likely that the pituitary content may not be determining cause of the ultimate dopamine response, but may itself be another result of a change in dopamine action that is induced by estrogen *in vivo*.

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