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The Influence of Estrogen on Pituitary Growth and on Prolactin Production *in Vitro* in the Diabetic Rat (40601)[1]

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It has been known since the 1930s that estrogen administration increased the size of the anterior pituitary (AP) (1, 2) and its content of prolactin (3), however the mechanism of this action is not fully understood. The action of estrogen appears to be directly on the pituitary because similar responses in pituitary growth and prolactin synthesis have been reported when estrogen was stereotaxically implanted into either the hypothalamus or the pituitary (4–6). Further, estrogen administration to animals with the AP transplanted either to the anterior chamber of the eye (7) or to the kidney capsule (8, 9) resulted in stimulation of pituitary growth and prolactin secretion. The addition of estrogen to the medium of AP explants has been reported to increase prolactin secretion by some investigators (10, 11) but not by others (12, 13). The effect of estrogen on pituitary growth *in vitro*, however, has been uniformally negative (11–13). The lack of agreement between the *in vivo* and the *in vitro* actions of estrogen on pituitary growth led to a search for the mitogenic factor *in vivo* that was absent *in vitro*. In other systems insulin has been shown to be mitogenic (14, 15). Thus, a study was undertaken to determine whether the estrogen-induced growth of the AP required the presence of insulin and whether insulin lack had any effect on prolactin production. This was accomplished by injecting estrogen into rats made diabetic by streptozotocin and measuring pituitary weight and the *in vitro* production of prolactin.

Materials and methods. Mature female Sprague–Dawley rats (Spartan Research Animals, Inc, Haslett, Mich.) initially weighing 200–220 g were housed two per cage in a room with controlled temperature (23 ± 2°C) and lighting (L14:D10; lights on 0600 to 2000 hr). The animals were acclimated to animal room conditions for 1 week before initiating the experiment.

The experimental design was initially to induce diabetes in ovariectomized (OVX) rats and then give estrogen followed by AP incubation to determine prolactin secretion. The duration of the experiments was approximately 24 days. A total of six groups were studied. One group was the intact control, sham injected; only organ weights and serum prolactin were obtained at the end of the experiment from this group. A second group was OVX and sham injected. A third group was OVX and 15 to 17 days later given a single sc injection of 0.5 mg polyestradiol phosphate (PEP—1 mg Estradurin, Ayerst lab., N.Y.); a single injection will keep animals in vaginal estrus for 4 to 5 weeks (16). A fourth group was OVX and 7 days later given a sc injection of 65 mg/kg BW of streptozotocin (Upjohn Pharmaceutical Co., Kalamazoo, Mich.) to induce diabetes. The fifth and sixth groups were OVX and 7 days later given streptozotocin. In one group insulin was immediately injected at a level of 2 IU/day (Ultralente, Eli Lilly and Co., Indianapolis, Ind.) for the duration of the experiment and PEP given 8–10 days later. In the other group 8 to 10 days after streptozotocin, when the animals were glycosuric and had a body weight loss of approximately 10 g, PEP was administered. The APs from the experiment were all incubated at the same time.

All animals were weighed and blood samples obtained by orbital sinus puncture following 3 min of ether anesthesia (17) immediately prior to the injection of streptozotocin (Day 7) and PEP (approximate Days 15–17). Blood samples at the end of the experiment (Days 22–24: 7 days after PEP) were obtained by decapitation after exposing the an-

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imals to 3 min of ether anesthesia. Some of the experimental groups were replicated for the purpose of obtaining AP weights and serum prolactin levels.

In a second experiment in which prolactin secretion rates were studied *in vitro* animals were OVX and immediately injected sc with either 5, 50, or 500 μg of estradiol benzoate (EB) for 3 days. At the end of this time the APs were removed and incubated simultaneously with APs from OVX and intact animals.

At the end of the experiment the AP was obtained by stunning the animal with a blow to the back of the head and decapitating it. The AP was removed from the posterior pituitary and divided into four pieces. Each AP was then weighed and placed into a glass scintillation vial containing 1 ml of Medium 199 (Microbiological Assoc. Inc., Bethesda, Md.) and 200 units/ml of penicillin and streptomycin. The tissue was incubated at 37°C in an Aquatherm Waterbath Shaker for 4 hr in a 95%O_2–5%CO_2 environment. At the end of

TABLE I. THE INFLUENCE OF STREPTOZOTOCIN AND ESTROGEN ON BODY WEIGHT (BW) AND BLOOD GLUCOSE LEVEL

Treatment[a]		Initial BW (g)[c]	Post-STR BW (g)	Post-STR Blood glucose (mg %)	Post-PEP BW (g)	Post-PEP Blood glucose (mg %)
OVX	(8)[b]	244 ± 7[a]	275 ± 7[a]	154 ± 12[a]	288 ± 12[a]	159 ± 6[a]
OVX, STR	(10)	233 ± 7[a]	223 ± 11[b, c]	585 ± 89[b]	219 ± 10[b]	589 ± 21[b]
OVX, STR, +IN, +PEP	(5)	254 ± 5[a]	242 ± 6[b]	633 ± 128[b]	254 ± 3[c]	454 ± 68[c]
OVX, STR, +PEP	(12)	236 ± 7[a]	203 ± 7[c]	530 ± 30[b]	206 ± 8[b]	620 ± 10[b]
OVX + PEP	(12)	235 ± 6[a]	267 ± 7[a]	159 ± 14[a]	264 ± 6[c]	161 ± 10[a]

[a] OVX = ovariectomized; STR = 65 mg/kg of streptozotocin. IN = insulin replacement, 2 IU/rat/day: PEP = 0.5 mg of polyestradiol phosphate.

[b] Numbers in parentheses indicate the number of animals per group.

[c] Different superscripts indicate significant differences among the values at that point in time ($P < 0.05$).

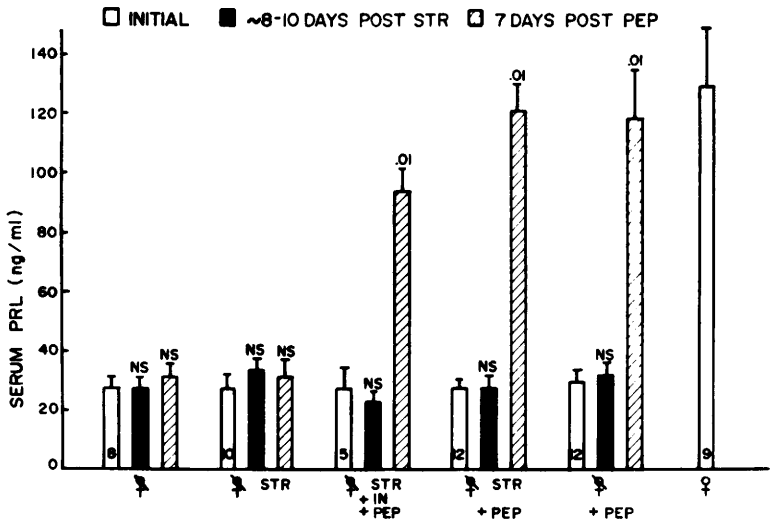

FIG. 1. The influence of streptozotocin and estrogen on serum prolactin (PRL) levels (mean ± SE of the mean). Blood samples were obtained following 3 min of ether stress. Initial: Blood samples obtained from the orbital sinus 1 week after ovariectomy and immediately prior to the injection of streptozotocin (STR). Post-STR: Blood samples obtained from the orbital sinus 8–10 days after the animals were injected with STR and immediately prior to the injection of PEP, i.e., approximately Days 15–17 of the experiment. Post-PEP: Blood samples obtained at the termination of the experiment by decapitation, 7 days after the injection of 0.5 mg PEP, i.e., approximately Days 22–24 of the experiment. The level of significance, compared to the initial value, is indicated above each bar; NS equals $P > 0.05$. Numbers within the open bars indicate the number of animals in each group.

incubation the AP tissue from each vial was homogenized in 3.0 ml of 1% triton X-100–PBS solution (18) using a glass tissue homogenizer. The 150-μl aliquots of incubation medium and pituitary homogenate were diluted 1:100 with the RIA buffer (1% BSA–PBS), frozen and stored at 20°C until assayed for prolactin using a specific double-antibody rat prolactin RIA at two dilutions in duplicate (19). Serum prolactin was determined by RIA at two dilutions in duplicate. Serum glucose levels were determined using a glucose oxidase method (Gluco State kit, General Diagnostics, Morris Plains, N.J.).

Statistical comparisons among groups were made using a one-way analysis of variance and Duncan's new multiple range test (20). The level of significance is indicated in the appropriate Table or Figure; a value of $P < 0.05$ was considered statistically significant.

Results. The ability of streptozotocin to induce diabetes was evidenced by a loss in body weight and a marked increase in the level of blood glucose (Table I). Insulin injection reduced the loss in body weight but did not alter blood glucose levels after the initial 8–10 days of injection. However, after an additional 7-day injection period, the blood glucose levels were significantly less than those of diabetic animals not receiving replacement therapy (Table I). Serum prolactin levels obtained under ether stress were not significantly different among animals not receiving estrogen (Fig. 1). The administration of estrogen resulted in an increase in serum prolactin similar to that of control animals regardless of whether the animal was diabetic or not (Fig. 1). The influence of estrogen in increasing uterine weight was not altered by the lack of insulin (Fig. 2A). However, in the diabetic animals estrogen administration failed to increase pituitary weight (Fig. 2B). When insulin was administered to the diabetic rats, estrogen was now able to stimulate

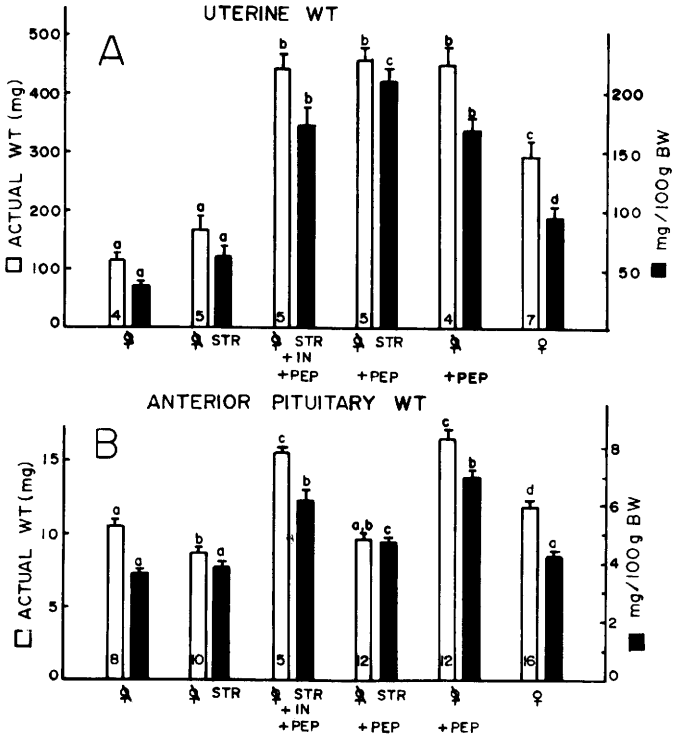

FIG. 2. The influence of streptozotocin and estrogen on uterine and anterior pituitary weight (mean ± SE of the mean). A: The open bars indicate the actual uterine weight while the solid bars indicate uterine weight per 100 g final body weight. B: The open bars indicate the actual anterior pituitary weight while the solid bars indicate the pituitary weight per 100 g final body weight. Groups with different superscripts above the bars are statistically different from each other: $P < 0.05$. Numbers within the open bars indicate the number of animals in each group.

an increase in AP weight comparable to that of nondiabetic animals (Fig. 2B).

When prolactin production was determined *in vitro* the diabetic animals administered estrogen appeared to produce considerably more prolactin than the nondiabetic animals when the prolactin level was presented on a per milligram of AP basis (Fig. 3B). However, if prolactin production is presented on a pituitary basis, then estrogen administration resulted in comparable prolactin values regardless of whether insulin was administered or not (Fig. 3A). This observation agrees very well with the serum prolactin results (Fig. 1). When estrogen is injected *in vivo* and the AP cultured *in vitro* the prolactin released into the medium on the basis of AP weight was significantly greater than that of AP from OVX animals (Fig. 4B). These data were comparable to those obtained from the experiment in diabetic rats when presented in the same manner (Fig. 4A). However, if one calculates the amount of prolactin released into the medium taking into consideration the concentration of prolactin in the AP, then the percentage released is comparable whether the AP was from an OVX, estrogenized, diabetic, or estrogenized diabetic animal (Fig. 4C,D).

Discussion. A single injection of streptozotocin is capable of inducing diabetes in the rat as evidenced by BW loss and serum hyperglycemia. By the end of the experiment the administration of insulin to the diabetic animal resulted in a body weight gain comparable to that of normal animals also receiving estrogen. The apparent inability of the exogenous insulin to maintain normal blood glucose levels is due, in all probability, to obtaining the serum sample 24 hr after the injection of insulin. Since blood glucose is capable of rapid and dynamic changes, more difficulty would be expected in demonstrating normal levels to an exogenous insulin injection than would be expected for body weight.

The inability of estrogen to increase AP weight in the absence of insulin has not, to our knowledge, been reported previously. Our data indicate (Fig. 2) that while insulin

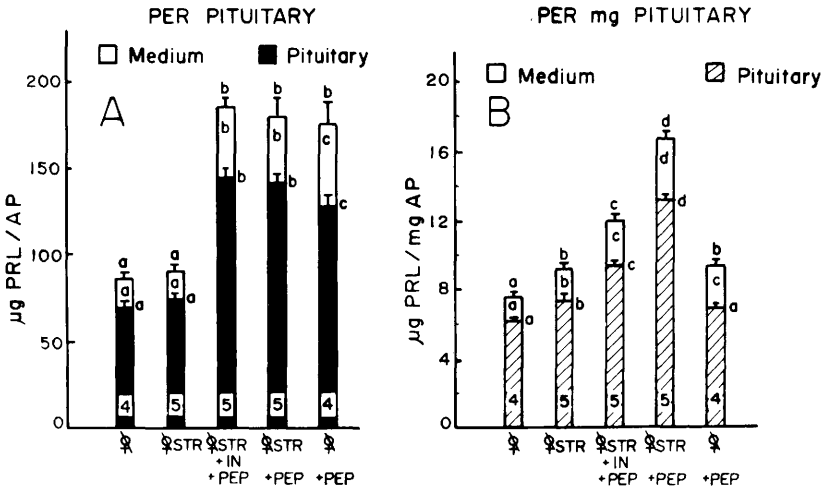

FIG. 3. The influence of streptozotocin and estrogen administration *in vivo* on prolactin (PRL) production *in vitro*. Prolactin levels were determined by radioimmunoassay. Total prolactin (medium plus pituitary content) is presented as follows: A: Prolactin is presented per pituitary as μg PRL/pituitary. The solid portion of the bar represents pituitary prolactin content while the open portion represents medium prolactin and B: Prolactin is presented per mg pituitary weight as μg PRL/mg AP/4 hr plus μg PRL/mg AP. The striped portion of the bar represents pituitary prolactin content, while the open portion of the bar represents prolactin found in the medium. Groups with different superscripts are statistically different from each other: $P < 0.05$. Superscripts above the bars represent comparisons of total prolactin while those within the open portion of the bar indicate differences among groups for the prolactin found in the medium, and differences in pituitary content among groups are denoted by different superscripts at the right side of the solid and striped bars. Numbers within the bars indicate the number of pituitaries per group.

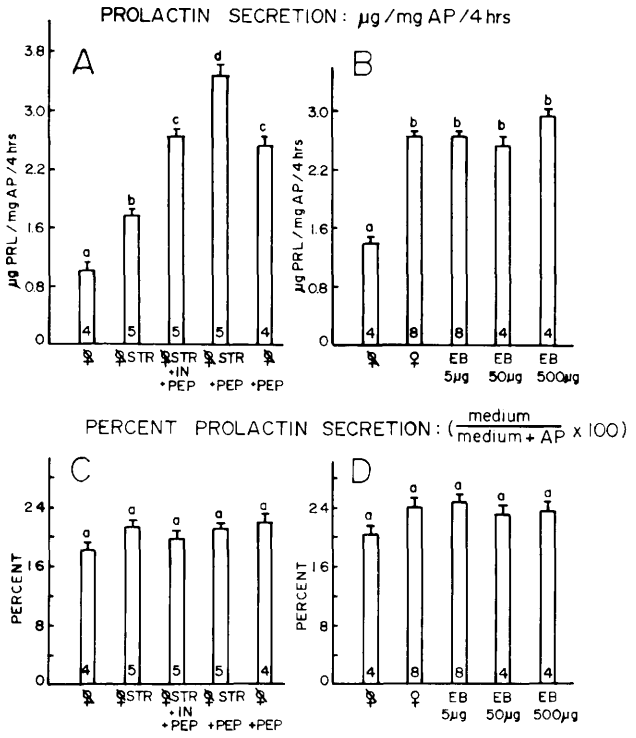

FIG. 4. The influence of polyestradiol phosphate (PEP) and estradiol benzoate (EB) injection to normal and diabetic rats *in vivo* on prolactin (PRL) secretion (release) *in vitro* (mean ± SE of the mean). The prolactin content of the medium was determined by radioimmunoassay and is presented as follows: In A and B as μg PRL/mg AP/4 hr and in C and D as a percentage of the total prolactin within the *in vitro* systems: (medium/medium + pituitary) × 100. Numbers within the bars indicate the number of pituitaries per treatment group. Groups with different superscripts above the bars are statistically different from each other; $P < 0.05$.

is not a requirement for the estrogen-induced increase in uterine weight it is necessary for the AP increase. Insulin therefore is an obligatory permissive factor in the mitogenic action of estrogen on the pituitary. Insulin has previously been reported to be mitogenic for liver growth (14, 15) and for mammary gland growth (21).

The method of presenting prolactin production *in vitro* is very important to the interpretation of the data. In our diabetic study, presenting the data on the basis of AP weight can lead to an incorrect interpretation. The data presented in this way would indicate a significantly greater release of prolactin into the medium by AP from diabetic animals receiving estrogen when compared to normal animals receiving estrogen (Figs. 3B, 4A). However, when one examines the circulating prolactin level in diabetic animals, they are comparable to normal animals. Care must be taken then when presenting *in vitro* prolactin production because under certain experimental conditions a disassociation between AP weight and prolactin production may occur. We have presented one such disassociation here.

The presence or absence of estrogen *in vivo* does not modify the proportion of prolactin released by the AP *in vitro*. What is greatly modified by estrogen treatment, however, is the secretory capacity of the AP. The estrogenized AP is capable of releasing more prolactin not because the percentage released is changed, but because it can release the same percentage from a much larger pool. What is significant to the physiology of the animal is the interplay between the capacity of the AP to secrete prolactin which in all probability is regulation by estrogen (17) and the release mechanism of the AP which is regulated by dopamine (22). When incubating the AP *in vitro* the inhibitory effects on the release mechanisms are all but eliminated and what

is examined is the secretory capacity of the gland. This consideration does not hold, however, if the regulator of the release mechanism, dopamine, is now added to the incubation system. In studies where this is done (23–25) it is now possible to examine factors which regulate prolactin release.

Summary. The influence of estrogen injection on anterior pituitary (AP) weight and on prolactin secretion *in vivo* and *in vitro* was examined in the diabetic rat. In the absence of insulin estrogen did not have any effect on increasing AP weight while the increase in uterine weight was similar to that of normal animals. Thus insulin is an obligatory permissive mitogenic agent for estrogen-induced pituitary growth. The lack of insulin had no effect on the estrogen-induced increase in prolactin production. However, the method of expressing prolactin production is significant to the interpretation. If the prolactin production is expressed on the basis of AP weight then erroneous conclusions can result, i.e., an increase in prolactin release. Only when prolactin production is expressed on a pituitary basis does the *in vitro* finding agree with the *in vivo* observation. Data are also presented under a number of experimental circumstances to indicate that the amount of prolactin released into the medium is a percentage (approximately 20–25%) of that present in the pituitary, and that estrogen influences the capacity of the pituitary to secrete prolactin *in vitro* in addition to an effect on the release mechanism *in vivo.*

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