Impaired Down-Regulation of Pituitary Dopamine Receptors by Estradiol in Aged Rats (42949)

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Abstract. **Administration of 17β-estradiol to mature (6–12 months) rats results in a more than 50% reduction in pituitary dopamine receptor concentrations, without affecting binding affinity. In contrast, when the same manipulation is performed on senescent (24–25 months) rats, negligible change in receptor concentration occurs. These results suggest that age-related increases in estrogen-stimulated prolactin release are not due to decreased dopaminergic inhibition at the receptor level.** [P.S.E.B.M. 1989, Vol 192]

Various investigators have reported that estrogen treatment decreases the number of available dopamine receptors in the pituitary (1–5). This effect occurs following both *in vivo* (1–4) and *in vitro* (5) administration of the hormone and, due to its relatively rapid onset, probably does not reflect a genomic action of estrogen. Pasqualini *et al.* (5) have recently proposed that estrogenic down-regulation of inhibitory dopamine receptors may be the mechanism by which the steroid stimulates pituitary prolactin release.

Our laboratory has frequently employed an aging model in attempts to elucidate signal transduction sequences for various hormone and neurotransmitter actions. In particular, we have observed that estrogenic stimulation of prolactin release actually increases with rat age (6) in contrast to estrogenic stimulation of lutenizing hormone release (6, 7) and many other steroid actions which decline during aging (for reviews see (8, 9)). If the hypothesis of Pasqualini *et al.* (5) is correct, we might predict that the estrogen-induced decrease in pituitary dopamine receptors might be greater in old rats, thus reducing inhibition of prolactin release more than in younger animals. Such a phenomenon would be extremely interesting in light of the fairly generalized decrease in response to steroid hormone observed during aging (8, 9). Moreover, this relatively rare type of age change might reflect a proposed membrane action of estrogen (5) in contrast to the more widely recognized genomic actions of the hormone (10). Thus, the aging process might differentially effect estrogen actions occurring through different signal transduction sequences.

For these reasons, we have examined the effects of *in vivo* estrogen administration on the concentrations of available pituitary dopamine receptors in rats of various ages.

Materials and Methods

Six- to 12- and 24- to 25-month-old female Wistar rats were obtained from the colony of the Gerontology Research Center. The endocrine and reproductive status of these animals has been described previously (11). All mature rats were cycling and all senescent animals were in constant diestrus. Animals with observable pituitary tumors or serum prolactin levels >200 ng/ml (essentially none of the younger and approximately 25% of the older rats) were excluded from the study. Some rats were bilaterally ovariectomized 3 weeks prior to use.

Rats were given four injections of 200 μg of 17β-estradiol suspended in 0.2 ml of cotton seed oil/kg body wt (or cotton seed oil only for controls) at 2-day intervals and sacrificed by decapitation 3 hr after the last injection. Trunk blood was collected for determination of estradiol and prolactin by radioimmunoassay (6) in some experiments. Anterior pituitary glands were removed and immediately frozen at −70°C. Pituitary tissue can be maintained in this way for at least 1 month without detectable loss of (D_2) dopamine receptors (12).

Membrane fractions were prepared from pituitaries by homogenizing tissue from three to six rats in each

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age group in 10 ml of Tris buffer (pH 7.4) in a Brinkman Polytron at setting 6 for 5 sec. After centrifugation at 50,000*g* for 10 min, the pellet was washed in the buffer mentioned above and recentrifuged as before. The final pellet was resuspended in 1.3 ml of 50 m*M* Tris buffer containing 0.1% ascorbic acid, 10 μM pargyline, 120 m*M* NaCl, 5m *M* KCl, 1 m*M* $CaCl_2$, and 1 m*M* $MgCl_2$, warmed to 37°C for 10 min, and returned to ice. Fifty-microliter aliquots of membrane suspension (0.25 mg) were incubated with ^{3}H-spiperone (specific activity, 22.0 Ci/mmol; New England Nuclear) in duplicate at concentrations of 0.25–2.0 $\times$ 10^{-9} *M* in a total volume of 400 μl. Specific binding was defined as that displaced by 10^{-6} *M d*-butaclamol. Membrane-bound radioactivity was trapped on Whatman GF/B filters and counted by liquid scintillation spectrometry.

^{3}H-Spiperone binding parameters (B_{max} and K_d) were obtained from Scatchard analyses (12) of saturation curves. Correlation coefficients for linear regressions were −0.9 or better in nearly all 34 individual plots. Values for receptor concentrations were corrected for membrane protein concentration as assessed by the method of Lowry *et al.* (14), using bovine serum albumin as a standard. Data were analyzed by Student's two-tailed *t* test (15).

Results and Discussion

Administration of estradiol to ovariectomized mature (6–12 months) rats results in an approximately 50% reduction in pituitary dopamine receptor B_{max} with no appreciable change in K_d (Fig. 1). In contrast, the same treatment of ovariectomized, senescent (24–25 months) rats has a minimal effect on both receptor parameters (Fig. 1).

Data from a number of such analyses of both intact and ovariectomized rats is displayed in Table I. Again,

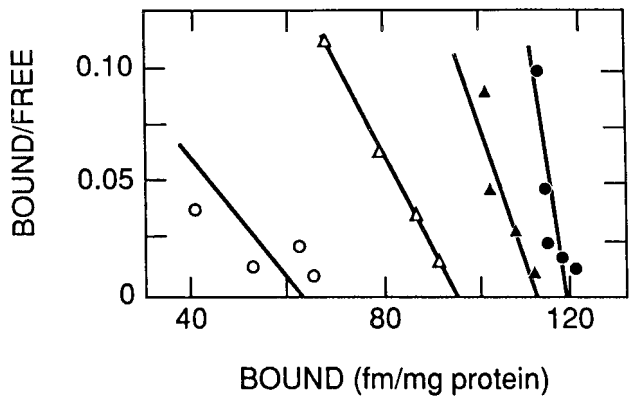

Figure 1. Effect of aging on estradiol-induced reduction in pituitary dopamine receptor concentrations. Six- and 24-month-old rats were injected with 17β-estradiol or vehicle and pituitary dopamine receptors determined as described in Materials and Methods. Each line represents a Scatchard plot from a membrane preparation of three to six pooled pituitaries. ●, 6-month-old vehicle treated; ○, 6-month-old estradiol treated; ▲, 24-month-old vehicle treated; △, 24-month-old estradiol treated.

Table I. Effect of Aging, Ovariectomy, and Estradiol on Rat Pituitary Dopamine Receptors

Age (months)	Receptor concentration (fmol/mg protein)		
	Intact	Ovariectomized	Ovariectomized + estradiol
6–12	129 ± 6 (8)[a]	135 ± 15 (3)[a]	63 ± 2 (4)[b]
24–25	140 ± 8 (6)[a]	148 ± 22 (6)[a]	118 ± 9 (7)[a]

Note. Animals were treated and pituitary dopamine receptors determined as described in Materials and Methods. Values represent the means ± SE for the numbers of pituitary membrane preparations (three to six individual pituitaries per preparation) indicated in parentheses.

[a] Not significantly different from each other ($P > 0.05$).

[b] Significantly different from all other groups ($P < 0.01$).

it can be seen that dopamine receptor concentrations are reduced by more than half following estradiol administration to mature rats. As has been previously reported (12), no age differences in pituitary receptor levels are seen as a function of age. This observation stands in marked contrast to the situation in the corpus striatum, in which many laboratories have documented age-associated loss in a variety of species (for a review see (16)) and an age-related increase in pituitary dopamine receptor density reported for Sprague-Dawley rats (17). Also, as shown in Figure 1, 17β-estradiol has very little effect on receptor concentrations in aged animals. Although not shown, no significant differences in K_d are observed for any group; all ranging from 0.1 to 0.3 n*M*. Age groups were initially analyzed as 6–8, 11–12, and 24–25 months. However, since no significant differences were observed between the two younger groups (6- to 8-month intact = 133 ± 9, 11- to 12-month intact = 127 ± 5; 6- to 8-month ovariectomized = 163, 11- to 12-month ovariectomized = 123 ± 13; 6- to 8-month ovariectomized + estradiol = 66 ± 2, 11- to 12-month ovariectomized + estradiol = 62 ± 2 fmol/mg protein), they were ultimately analyzed as one 6- to 12-month-old group to improve statistical accuracy. It was not possible to detect differences in receptor concentrations between intact and ovariectomized rats as have been reported by others (18, 19). This may be due to the fact that pooled pituitary membrane preparations from randomly cycling young animals were used, thus minimizing estrogen-dependent cycle differences. Old animals were essentially all in constant diestrus, but appeared to be much less sensitive to estrogen than young counterparts. Nevertheless, it is quite clear that the relatively higher concentrations of estradiol employed in the present protocol are sufficient to decrease receptor concentrations.

Since age differences in circulating estrogen and/or resultant prolactin levels (6, 11) might differentially affect dopamine receptor concentrations, both hormone levels were determined in plasma of control and estradiol-treated 6- and 24-month-old ovariectomized

rats. Table II shows that elevated plasma estradiol levels after treatment are not significantly different between mature and senescent rats. In contrast, circulating prolactin levels are much higher in the sensecent than mature group, both before and after estradiol treatment as has been shown previously (6). Since control and treated animals were separate groups, large variation in prolactin levels of aged animals (6) precluded detection of a significant effect of estradiol in this group.

Taken together, the present results suggest that, like many other steroid actions (8, 9), estradiol-induced reduction in pituitary dopamine receptor concentrations is markedly reduced during aging. Our findings of a more than 50% reduction in receptor concentrations of mature animals, without altered binding affinity, are in close agreement with those of numerous other laboratories (1–5). Since the mechanism involved in such down-regulation remains unclear, it is difficult to predict at what level the age-associated alteration might occur. Certainly, loss of steroid receptors and/or reduced nuclear association of receptor-steroid complexes have been implicated in many cases of impaired steroid responsiveness during aging (8, 9). However, since the present phenomemon has been reported to occur *in vitro* with a much shorter time course than *in vivo* (5), classical intracellular receptor mechanisms may not be involved. Instead, it is possible that estradiol may be acting directly at the plasma membrane.

Alternatively, despite the fact that circulating prolactin levels are greatly elevated in aged rats (6), both before and after estradiol treatment, Sarkar *et al.* (20) have shown that elevated prolactin exerts a short loop feedback effect, increasing hypothalamic dopamine turnover in young rats but not old. Thus, it is possible that this increased dopamine turnover is responsible for the preferential effect on dopamine receptors in young rats.

Another conclusion from the present study is that age-related increases in basal and estrogen-stimulated pituitary prolactin release (6) are not due to greater down-regulation of inhibitory dopamine receptors in old rats. Although our data do not directly contradict the hypothesis of Pasqualini *et al.* (5) that estrogenic stimulation of prolactin secretion may be at least partially due to desensitization of pituitary cells to dopamine in young rats, they do suggest that age changes in control of secretion are not due to alterations at the dopamine receptor level. We have, however, previously shown that direct dopaminergic inhibition of prolactin release from cultured pituitary cells is indeed reduced with age (12). In concert with the present findings, this reduction is not due to age changes in dopamine receptors.

It seems clear that aging may differentially affect both dopaminergic and estrogenic regulatory mechanisms as well as interactions between the two systems. Resolution of the questions raised here will be dependent on further elucidation of the signal transduction sequences involved in both systems.

Table II. Effect of Aging and Estradiol Treatment on Circulating Levels of Estrogen and Prolactin

Age (months)	Estrogen (pg/ml)		Prolactin (ng/ml)	
	Control	Estradiol treated	Control	Estradiol treated
6	24 ± 8^a	764 ± 166^b	4.5 ± 0.5^c	10.3 ± 2.8^d
24	18 ± 2^a	664 ± 268^b	100.0 ± 42.2^c	88.2 ± 28.8^d

Note. Trunk blood was collected from control (cotton seed oil) or 17β-estradiol-treated, ovariectomized rats and prolactin and estrogen levels were determined by radioimmunoassay as described previously (6). Values represent the mean ± SE for five to six animals in each group.

[a,b] Not significantly different from same letter group ($P > 0.05$).

[c,d] Significantly different from same letter group ($P < 0.05$).

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