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Effects of Dopamine, Norepinephrine, and Serotonin on Plasma Prolactin Levels in Ovariectomized, Pituitary-Grafted Rats[1] (40100)

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The ability of the catecholamines to affect prolactin (Prl) release from the anterior pituitary (AP), both *in vitro* and *in vivo,* has been studied. Shaar and Clemens (1) demonstrated that norepinephrine (NE) and dopamine (DA), when administered *in vitro* in amounts not greater than that found in the hypothalamus, inhibited Prl release. This suggests a direct effect of these amines on the AP. This confirmed earlier *in vitro* work (2, 3), which indicated that several concentrations (10^{-6} *M*–10^{-4} *M*) of the catecholamines could inhibit Prl release. Kamberi *et al.* (4), using an *in vivo* system, concluded that neither DA nor NE affected Prl release by a direct action on the AP. They suggested that the effects of the catecholamines on Prl release were mediated through the hypothalamic–hypophyseal complex, with DA being more effective than NE in reducing plasma Prl. In direct contrast to this, Takahara *et al.* (5) demonstrated a lowering of plasma Prl following injection of NE or DA into the hypophysial portal system, indicating a direct effect of these amines on the AP. Blake (6) further demonstrated that DA, when infused intravenously, could significantly lower basal plasma Prl levels and block the spontaneous surge of Prl in plasma seen during proestrus. He could not conclude, however, whether the inhibitory effects of DA were directly on the AP or mediated through the median eminence of the hypothalamus.

Serotonin (5-HT), on the other hand, is increasingly implicated as a stimulator of Prl release. Lawson and Gala (7) demonstrated an increase in Prl release following systemic administration of 5-HT in ovariectomized, estrogen treated rats. 5-HT, injected into the third ventricle, also has been shown to stimulate Prl release (8). Parachlorophenylalanine, a drug used to chronically block 5-HT synthesis, inhibited the suckling induced rise in plasma prolactin (9). This drug also was found to decrease serum Prl levels in estrogen primed, ovariectomized rats (10). MacLeod, using an *in vitro* system, has shown no direct effect of 5-HT on Prl secretion from the AP (11).

The present experiments were designed to investigate further if amines have a direct effect on the AP secretion of Prl. Experiments utilized the ovariectomized rat with intact pituitary and an additional AP transplanted under the kidney capsule.

Materials and methods. Female, Sprague–Dawley rats (Laboratory Supply, Indianapolis, IN) were housed in a temperature (25 ± 2°) controlled room with lights on from 0500 to 1700 hr. Rats were given free access to rat chow and water. All rats were bilaterally ovariectomized. One week following ovariectomy, donor rats were killed by decapitation, the anterior lobe was separated from the posterior lobe and immediately transplanted under the right kidney capsule of the host rats (1 AP/rat). One week following pituitary transplantation, an indwelling aortic cannula was inserted via the right carotid artery under ether anesthesia using the method of Gay *et al.* (12). This cannula was then used for both the infusion of biogenic amines as well as for blood sampling the next day in awake and unrestrained rats.

The following compounds were utilized for infusions: L-norepinephrine bitartrate (lot number 510-1940) and dopamine HCl (lot number 930-1370) from Sigma Chemical, St. Louis, MO, and serotonin creatinine sulfate complex hydrate (lot number 901192) from Calbiochem Inc., Los Angeles, CA. Each amine was dissolved in a phosphate buffered saline (PBS)-ascorbic acid solution (0.02% ascorbic acid). The concentration of the biogenic amines in solution was 1 μg per 10 μl PBS-ascorbic acid adjusted to a final pH of 5.5 with 0.1 *N* HCl. This PBS-ascorbic acid solution, devoid of any biogenic amines, was utilized as a control solution at pH 5.5. All

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solutions were prepared fresh immediately before use and were kept on ice throughout the infusion procedure. In addition, all solutions were used only once; fresh solutions were made for each infusion.

Infusions were carried out with the aid of an LKB peristaltic pump (LKB-Produkter, Bromma, Sweden). The rate of infusion was kept constant at 10 μl per min throughout the experiment except for 20–30 sec interruptions when blood was withdrawn. A preinfusion blood sample was taken to establish basal Prl levels and the infusion was then begun. Samples were taken at 5, 10, 20, 30, 40, 50 and 60 min following the commencement of infusion. The infusion was terminated at 60 min and a final blood sample was taken 60 min later.

The Prl concentration of all blood samples was determined via a double antibody radioimmunoassay for rat prolactin (13). Purified rat Prl from NIAMDD was used for iodination and NIAMDD-RP-1 was used as a reference standard. Antibodies against rat prolactin were obtained from J. D. Neill (Emory University). A two-way analysis of variance and Dunnett's *t* test were used to compare plasma Prl levels between control and amine infused groups, as well as between the preinfusion sample and the postinfusion samples within each group.

Results. The results of this study are shown in Table I. The PBS infused homografted group showed a significantly higher basal plasma Prl level than the PBS infused group without any AP transplants. Assuming the half life is the same for Prl secreted from each pituitary site, at least 85% of plasma Prl may be due to the AP transplant. Infusions of both NE and 5-HT had no significant effect on Prl secretion when compared both to the PBS infused homografted group and to the preinfusion level within each respective group. Dopamine, however, was able to suppress Prl release by 5′ of infusion, and this decreased plasma Prl level was significant by 20′ when compared to the preinfusion levels. When compared to the PBS infused, homografted controls, plasma Prl levels were significantly suppressed after 5′ of infusion. By 40′ of DA infusion, plasma Prl levels were at their minimum, and remained basal until the infusion was stopped. This plasma Prl level was not significantly different from the levels seen in the PBS infused animals without pituitary transplants. After 1 hr following the cessation

TABLE I. EFFECT OF INFUSION OF DA, NE, AND 5-HT ON PLASMA PRL LEVELS IN OVARIECTOMIZED-AP TRANSPLANTED RATS.

	Prolactin (ng/ml)[b]								
Treatment	Pre[c]	5′	10′	20′	30′	40′	50′	60′	120′
OVX No Transplant PBS (5)[a]	16.5 ±9.2	13.1 ±5.9	7.4 ±1.5	12.9 ±4.0	7.8 ±1.1	9.1 ±1.4	10.9 ±4.1	7.8 ±0.1	9.4 ±0.5
OVX AP Transplant PBS (9)	93.3 ±11.7	94.1 ±11.1	85.2 ±9.5	94.8 ±9.1	80.4 ±7.9	90.2 ±8.5	84.6 ±6.4	82.2 ±6.2	94.9 ±6.8
OVX AP Transplant DA (7)	78.3 ±13.7	56.2 ±12.9[d]	43.9 ±13.9[d]	31.1 ±8.9[d, e]	23.9 ±7.9[d, e]	20.2 ±4.6[d, e]	20.6 ±4.6[d, e]	19.8 ±4.5[d, e]	97.8 ±26.2
OVX AP Transplant NE (7)	83.0 ±17.9	81.0 ±20.0	81.3 ±21.3	65.0 ±14.5	67.7 ±17.5	88.8 ±24.7	79.2 ±20.4	98.2 ±28.0	96.4 ±26.8
OVX AP Transplant 5-HT (8)	82.6 ±12.4	79.6 ±9.6	81.1 ±11.9	83.3 ±11.2	80.4 ±8.6	85.8 ±10.4	77.4 ±8.7	86.3 ±12.4	75.0 ±8.2

[a] Number of rats per group.
[b] Mean ± SE of the mean.
[c] All infusions began after the first sample was obtained and ended at 60′.
[d] Significantly different from PBS infused, homografted controls ($P < 0.05$).
[e] Significantly different from preinfusion levels ($P < 0.05$).

of DA infusion, plasma Prl levels returned to their preinfusion level.

Discussion. Isolated AP homografts, placed under the kidney capsule, are known to secrete tonically large amounts of Prl, presumably due to the removal of the inhibitory influence of the hypothalamus (14). This source of Prl accounts for the large difference in plasma Prl seen between the PBS infused homografted group and the PBS infused nontransplanted group. The suppression of plasma Prl levels seen during DA infusion indicate the ability of DA to directly suppress Prl release from an AP graft, *in vivo.* This effect was pronounced and had a rapid onset. It had been previously shown that systemic intravenous infusions of DA could depress basal plasma Prl levels in the intact, cyclic rat (6). While it is likely that some of the suppression of Prl release observed in our work may be occurring in the intact pituitary, the amount of suppression seen certainly demonstrates an inhibitory effect of DA directly on the isolated pituitary graft. If Prl secretion from the intact pituitary were to completely cease and no suppression of Prl release from the AP graft occurred, the resultant decrease in plasma Prl levels would be expected to be far less than that observed. It has been shown that rats previously hypophysectomized have detectable plasma PIF levels whereas intact controls do not (15). An intact hypothalamic–pituitary axis was present in our model, preventing the release of detectable amounts of PIF into the systemic blood. Injections of L-dihydroxyphenylalanine (L-Dopa) have been shown to increase Prl release inhibiting activity of systemic blood in hypophysectomized rats, presumably through stimulation of hypothalamic PIF (16). The use of DA rather than L-Dopa in our studies, however, precludes the large scale movement of this amine into the hypothalamus. These results demonstrate the ability of DA to directly and dramatically suppress Prl secretion from the anterior pituitary, *in vivo,* and are consistent with the view that DA may be PIF. However, this study does not eliminate the possibility that DA normally acts by stimulating release of another kind of PIF from the hypothalamus. These results also provide an animal model which is useful for studying agents which have a direct action at the AP on Prl secretion.

Norepinephrine at a dosage of 1 μg per min appeared to exert no effect on Prl release from either the pituitary graft or the intact pituitary. Higher doses were not used because of possible systemic effects of the NE. This seems consistent with the findings of Blake (6), who suggested that the partial suppression of the Prl surge on the afternoon of proestrus in the intact rat seen after two hours of systemic infusions of NE was a nonspecific effect. Serotonin also appeared to exert no effect on either pituitary. These findings are in agreement with the *in vitro* work of MacLeod, who found no direct effect of 5-HT on an isolated anterior pituitary (11). This does not refute the findings of those who observed an increase in Prl release following systemic injection of 5-HT (7), since 5-HT was administered in that study to estrogen-treated ovariectomized rats as a massive bolus injection. No steroids were administered in the present study.

Summary. Chronically ovariectomized rats with intact pituitary glands were implanted with anterior pituitary homografts under the kidney capsule. Rats which received a transplanted anterior pituitary exhibited significantly higher basal plasma prolactin levels than rats receiving no transplants. Rats received systemic infusions of norepinephrine, dopamine, and serotonin. Only dopamine was capable of significantly suppressing prolactin secretion from the pituitary homograft. Norepinephrine and serotonin had no effect on plasma prolactin levels. These results demonstrate the direct effect of dopamine on prolactin release by the anterior pituitary and are consistent with the view that dopamine may be PIF.

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