Intrahypothalamic Pituitary Grafts Elevate Prolactin in the Cerebrospinal Fluid and Attenuate Prolactin Release following Ether Stress (43161)

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Abstract. **Anterior pituitary (AP) tissue grafted into the hypothalamus of female rats inhibits the luteotrophic prolactin (PRL) secretion which normally follows mating. Dopamine blockade has been shown to overcome this inhibition, suggesting that the grafts suppress PRL release from the *in situ* pituitary by the action of graft PRL increasing dopamine activity in the hypothalamus.**

To examine whether PRL levels in the cerebrospinal fluid (CSF) were elevated by the AP grafts, CSF samples were taken from 5 control rats and 10 rats bearing intrahypothalamic AP grafts. Mean PRL concentrations in the CSF of the control rats were 3.0 ± 0.8 ng/ml. The grafted rats had significantly higher concentrations of PRL in their CSF, averaging 23.2 ± 4.2 ng/ml ($P < 0.005$). Plasma PRL concentrations were similar in the control and grafted rats.

PRL release in response to 5 min of ether stress was examined in 8 control and 11 grafted rats. In control animals, PRL rose from 4.2 ± 1.5 to 44.7 ± 9.0 ng/ml following exposure to ether, but the response was significantly attenuated in the grafted rats, peaking at 9.3 ± 1.4 ng/ml ($P < 0.001$). This inhibition of response due to the grafts was evident within 1 week of graft placement.

The results confirm that the presence of intrahypothalamic AP grafts led to the accumulation of supranormal PRL concentrations in the CSF. This elevated PRL suppressed pituitary PRL release in response to ether stress, probably by an autoregulatory feedback activation of the inhibitory tuberoinfundibular dopaminergic neurons in the hypothalamus. [P.S.E.B.M. 1991, Vol 196]

Rats bearing anterior pituitary (AP) tissue grafts in the hypothalamus have normal 4- to 5-day estrous cycles, but do not become pregnant when mated (1). It was shown (2) that these rats do not exhibit the nocturnal and diurnal luteotrophic prolactin (PRL) surges which normally follow coitus. Because dopamine (DA)-blocking drugs for 3 to 4 days after mating reversed the graft-induced sterility, it was suggested that the PRL surges were inhibited by the local action of PRL from the grafts stimulating the activity of the tuberoinfundibular dopaminergic (TIDA) neurons (3), in the same manner as occurs in short loop feedback or autoregulation with hyperprolactinemia (4).

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This article reports that intrahypothalamic AP grafts caused significant elevation of PRL in the cerebrospinal fluid (CSF), and that rats with such grafts showed significant attenuation of the PRL response to ether stress.

Materials and Methods

One half to one AP from 6- to 12-day-old pups was grafted into the anterior hypothalamus near the paraventricular nuclei in females of an inbred strain of Wistar rats, as described earlier (1, 3). Rats were housed at 23 ± 1°C with lights on daily between 0600 and 1800 hr. Daily vaginal smears were taken before and after mating with fertile males, and rats were used in subsequent studies only if their cycles continued uninterrupted following fertile matings on 2–4 consecutive estrous periods, demonstrating the antiluteotrophic effect of the grafts. At the end of the study all brains were serially sectioned as described previously (5) and examined for the presence and location of the AP grafts.

CSF was collected from 5 control rats and 10 rats bearing intrahypothalamic AP grafts as described elsewhere (6). The samples were taken under ether anesthesia and collection took approximately 15 min, after which a terminal blood sample was taken by heart puncture. These procedures were performed between 1000 and 1100 hr regardless of the stage of cycle to avoid the times of physiologic surges of PRL (7). Both CSF and plasma were stored at −20°C until assay.

For ether stress studies, an indwelling jugular cannula was surgically implanted as described earlier (8) into 8 control female rats and 11 rats bearing functional 3-month-old AP grafts. Cannulas were implanted on the day of proestrus or estrus, at least 48 hr prior to the experiment. Between 1000 and 1300 hr on the day of diestrus, these rats were placed in an ether box saturated with ether vapor for 5 min. Blood samples (0.4 ml) were collected before exposure to ether, then 5, 10, 15, 30, and 60 min after the start of the ether exposure. Blood volume was replaced with sterile 0.9% NaCl immediately after sampling. The samples were centrifuged immediately, and the plasma was stored frozen at −20°C until assay.

To determine how soon after implanting the grafts the ether stress response was inhibited, single blood samples were taken directly from the jugular vein of a second group of rats after 5-min exposure to ether at 7, 14, or 21 days after AP tissue had been grafted into the hypothalamus. Seven control rats had a single blood sample taken using the same method for comparison.

PRL concentrations in the plasma and CSF samples were assayed separately by radioimmunoassay using materials provided by the NIDDK through its National Hormone and Pituitary Program (University of Maryland School of Medicine). The method has been described previously (8). As a polyethylene glycol separation step was used, assay for plasma samples included PRL-free plasma with the standard dilutions of reference preparation rPRL-RP-3 to keep protein levels relatively constant. No plasma was added to the standards for the CSF assay. Inter- and intraassay coefficients of variation for the plasma PRL assay were 10.8 and 5.2%, respectively. All CSF samples were measured in a single assay, with the intraassay coefficient of variation of 7.0%. Results are expressed in terms of ng/ml rPRL-RP-3.

Results

Pituitary tissue of neonates was grafted in the hypothalamus of 25 female rats. All grafted rats had 4-day cycles, and when joined with fertile males two failed to mate and four became pregnant. The remaining 19 mated at 2–5 consecutive estrous periods without becoming pregnant or prolonging the cycles and were used for subsequent studies. At postmortem examination, all rats used had AP grafts embedded in the neural tissue 1- to 1.5-mm lateral to the midline. The grafts were between 1.0-mm anterior and 2.0-mm posterior to the paraventricular nuclei, and either dorsal or ventral to them. There was no clear relationship between the size or position of the grafted AP and the eventual PRL concentration in the CSF.

CSF. Mean PRL content in the CSF and the plasma sample following CSF collection from control rats and from rats bearing intrahypothalamic AP grafts are shown in Table I. Although the mean plasma PRL values are similar for the two groups of rats, the CSF values are highly divergent. The PRL concentration in the CSF of 5 control animals was 3.0 ± 0.8 ng/ml compared with CSF in 10 grafted rats which contained an average of 23.2 ± 4.2 ng/ml, significantly more than the control group (unpaired t test, $P < 0.005$). In six of nine grafted animals, the PRL concentration in the CSF exceeded that in the plasma of the same rat.

Ether Stress. The effect of 5-min exposure to ether on plasma PRL levels was tested in 8 control rats and 11 rats bearing intrahypothalamic AP grafts (Fig. 1). In the unoperated control rats, PRL rose from baseline levels of 4.2 ± 1.5 ng/ml to peak at 44.7 ± 9 ng/ml immediately following 5-min exposure to ether. PRL then fell to 19.9 ± 2.8 ng/ml at 10 min and had returned to basal values by 30 min. By contrast the rats with AP grafts in the hypothalamus had much lower PRL at 5 and 10 min (9.3 ± 1.4 and 7.5 ± 1.1 ng/ml, respectively) and returned to basal values by 15 min. Although the response to ether was very significantly reduced in the grafted rats compared with the controls (unpaired t test, $P < 0.001$), it was still significantly elevated above

Table I. Individual PRL Concentrations in the Plasma and CSF of Control Rats and Rats Bearing Intrahypothalamic AP Grafts

	Plasma PRL (ng/ml)	CSF PRL (ng/ml)
Controls	49.5	5.5
	24.4	4.5
	17.9	1.6[a]
	10.0	1.6
	7.4	1.6
Mean	21.8 ± 7.5	3.0 ± 0.8
Grafted	34.9	19.0
	30.1	27.3
	29.2	39.7
	24.5	12.5
	23.8	44.1
	11.1	17.7
	8.0	27.8
	5.5	10.5
	3.5	9.8
	—[b]	21.1
Mean	19.0 ± 4.0	23.2 ± 4.2[c]

[a] Limit of sensitivity of CSF assay.
[b] Plasma sample lost.
[c] Significantly different from control, $P < 0.005$, unpaired t test.

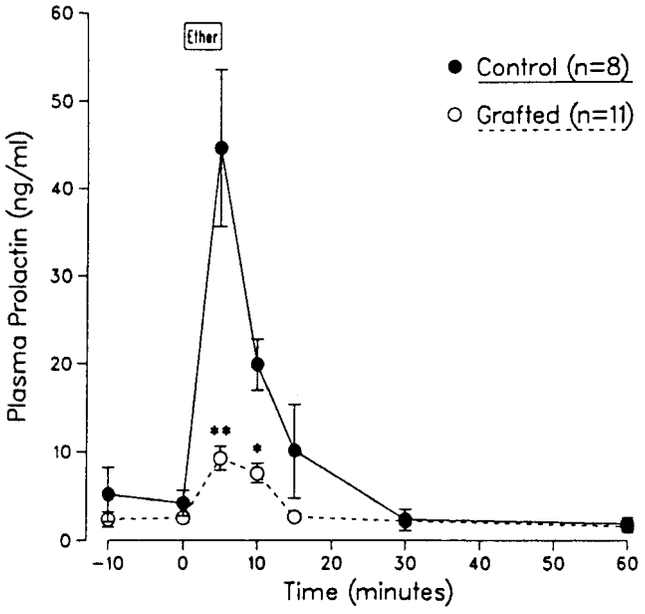

Figure 1. Changes in plasma PRL following exposure to 5 min of ether in control rats and in rats bearing intrahypothalamic AP grafts (grafted). ** $P < 0.001$, * $P < 0.05$ compared with controls (unpaired t test).

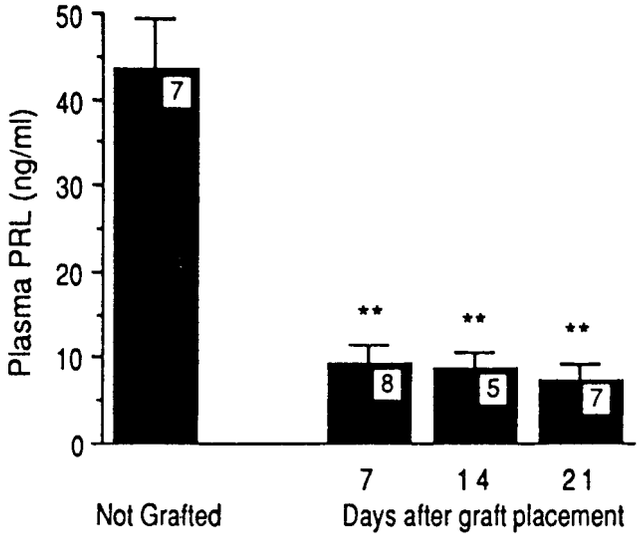

Figure 2. Plasma PRL in samples taken after 5-min exposure to ether in control animals and at 7, 14, or 21 days after grafting AP tissue into the hypothalamus. ** $P < 0.001$ compared with nongrafted animals (unpaired t test). The number of rats in each group is shown inside the bars.

baseline values (paired t test, $P < 0.001$). Basal PRL values were not significantly different between grafted and control rats.

Figure 2 shows plasma values from single blood samples taken from the jugular after 5-min ether stress in control rats and in rats at 7, 14, and 21 days after grafting AP tissue into the hypothalamus. Plasma PRL was significantly less in all three groups of rats bearing intrahypothalamic grafts than it was in the controls. There was no difference between the groups so that grafts were as effective after 1 week as they were after 3 weeks.

Discussion

In rats bearing intrahypothalamic AP grafts, PRL released spontaneously from the graft was found in unusually high amounts in the CSF, averaging more than 20 ng/ml. Gonzalez *et al.* (9) found even higher concentrations of PRL in the CSF 1 week after pituitary tissue was injected into the lateral venticle. Our grafts were located at least 1–1.5 mm from the third ventricle in the tissue surrounding the paraventricular nucleus, which may be why the PRL concentration in our CSF samples were considerably lower than theirs. Despite these differences in absolute values, in both studies PRL concentrations in the CSF of animals bearing pituitary tissue within the brain were significantly higher than in control animals (Table I). This PRL in the CSF could not have been of plasma origin in the absence of a significant concentration gradient.

PRL in the CSF has been shown to follow changes in plasma PRL but always at considerably lower concentrations (6). In normal cycling rats, PRL concentrations in the CSF were usually less than 2 ng/ml (10). PRL values in the CSF from control rats in our study were in good agreement with this even though we collected CSF under ether anesthesia as opposed to urethane used in the previous study (10). In pseudopregnant rats, CSF PRL concentrations increased twice a day at the same time as the luteotrophic plasma PRL surges, with maximum values of approximately 20 ng/ml obtained at 0500 hr during the nocturnal surge, when plasma PRL exceeded 100 ng/ml (10). CSF PRL concentrations were always considerably below comparable plasma values in control rats but in rats with intrahypothalamic AP grafts, PRL was at higher concentrations in CSF than in plasma in six of nine rats. The mean CSF PRL concentration in the grafted rats was as high as the maximum reported concentrations in control animals during the nocturnal surge (10).

PRL has long been known to autoregulate its own secretion, the so-called "short loop" feedback (11). PRL has been shown to activate the TIDA neurons directly following systemic (4, 12) or intracerebroventricular (4, 13) administration, to elevate levels of DA in the portal blood (14), and to reduce pituitary PRL secretion when infused into the CSF (4) or injected subcutaneously (15). PRL from pituitary tissue injected into the third ventricle also increased DA activity in the median eminence (9). We have shown above that intrahypothalamic AP grafts elevate PRL concentrations in the CSF which might activate the TIDA neurons as shown in other studies. We earlier suggested this as a mechanism to explain the absence of luteotrophic PRL surges in these grafted rats (3). While the grafts may continue

to secrete PRL, very little of this could enter the general circulation (16), and plasma PRL would remain low.

We have also shown that intrahypothalamic AP grafts greatly attenuated the elevation of plasma PRL observed following exposure to ether stress. Stress elevates plasma PRL by reducing activity of the inhibitory TIDA neurons in the median eminence (17, 18), which appears to be mediated by opioid and serotoninergic neurons (17). There is also considerable evidence that the stress-induced release of PRL may involve one or more PRL-releasing factors (PRF). Ether stress-released PRL after all DA had been depleted by reserpine (19) or after total blockade of DA receptors (20). Electrolytic lesions destroying the paraventricular nucleus prevented the release of PRL induced by ether stress, even though the TIDA system was intact (21), suggesting that inhibition of the DA system in response to ether stress is only a contributory factor to the release of PRL induced by PRF originating in the paraventricular nucleus. Assuming the PRL from the grafts has elevated DA in the median eminence (4, 9, 12–14), our results suggest that withdrawal of DA inhibition is necessary to allow PRL release in response to ether stress. The small but statistically significant rise in PRL observed in the grafted rats after exposure to ether as described above may be evidence of some PRF activity partially overriding DA inhibition. Whether PRL from the intrahypothalamic AP grafts concomitantly reduced the secretion of one or more PRF was not answered by the present study.

Although in our ether stress study there was no significant difference between basal levels of PRL in grafted and ungrafted control rats, previous experiments have shown that basal PRL secretion is reduced by intracerebro-ventricular infusion (4) or subcutaneous injection (15) of PRL. The absence of such an effect in the present experiment may simply reflect the small number of animals used. Over a number of studies using rats bearing intrahypothalamic AP grafts we have observed that basal levels are consistently lower in grafted rats than in controls (2), and the combination of data from several studies has shown significant depression of basal secretion in the grafted animals (our unpublished data).

The depression of the ether stress response by the grafts was evident within 7 days of graft placement and was still present when tested 3 months later. The latter observation is not surprising as the blockade of the luteotrophic surges by these grafts confers lifetime sterility on the rats (1, 22). However, it is interesting that intrahypothalamic AP grafts terminated pregnancy in only 2 of 11 rats when implanted on the day after mating (22), whereas blocking the luteotrophic PRL surges by implantation of PRL (23) or placental lactogen (24) into the hypothalamus terminated pregnancy within days. Although inhibition of the PRL increase after exposure to ether was possible within 7 days, the coitus-induced surges apparently were not blocked in the first seven days after grafting. A more prolonged exposure to enhanced DA may be necessary to block the coitus-induced surges of PRL as they rely on different PRF from the ether response (25, 26) and probably also require quantitatively different levels of DA withdrawal.

The similar plasma concentration of PRL between control and grafted rats following CSF collection warrants some comment. Under ether anesthesia, it was expected that the plasma PRL would be higher in the controls than in the grafted rats, as in our specific ether experiment. There are several possible explanations for this discrepancy. The collection of CSF samples required that the animal be placed in a stereotaxic instrument and undergo surgical procedures for approximately 15 min. Blood collection by heart puncture following this CSF collection must be more stressful than 5-min exposure to ether alone and could possibly have overcome the graft inhibition. The PRL response to ether stress is usually quite short, peaking after 5 min and even if exposure to ether is continued, PRL concentrations return to baseline values by 30 min (21). While the PRL-releasing effect of ether over the duration of CSF collection may not have been substantial, the unknown additional stress of the surgery may have caused some reduction of DA and therefore some elevation of PRL in both groups of animals. Demarest *et al.* (18) have shown that 30 min of restraint stress, but not mild ether stress, can reduce DA activity in the median eminence even after DA has been stimulated by hyperprolactinemia induced by intraperitoneal injection of ovine PRL, and our result is of the same pattern.

AP grafts in the hypothalamus led to the accumulation of supranormal concentrations of PRL in the CSF. This PRL does not enter the plasma and in some way, probably by enhancement of TIDA neuronal activity, prevented the *in situ* pituitary from exhibiting the surges of PRL which normally follow coitus (2) and the more acute rise in PRL which follows ether exposure. It would be of interest to determine whether the intrahypothalamic AP grafts also impair the secretion of PRF into the portal blood.

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