may result from Raman scattering from vibrational hot bands which are thermally populated in anharmonic potentials of low energy such as that of the iron-histidine bond (~ 10 kcal) (*4*).

The changes on going through the melting transition are very striking and bring about the distinction in the iron-histidine mode between the two quaternary structures. The other surprising effect is the appearance of a line at 160 cm^{-1} that is absent below 0°C. At room temperature this mode is absent in myoglobin but present in HbA (*15*), β_4 tetramers, and α chains. Its normal mode origin has not been assigned. When the excitation frequency is changed to 4131 Å it becomes exceedingly strong in comparison to other modes in the spectrum. The changes in frequency (5 to 10 cm^{-1}) and intensity of this mode with changing quaternary structure (*6*), which parallel the behavior of the iron-histidine mode, strongly suggest that the 160 cm^{-1} mode also involves the proximal histidine. A definitive determination of the origin of this mode must await more extensive studies. Accompanying the appearance of the 160 cm^{-1} mode is the development of a quaternary structure dependent difference in the iron-histidine stretching mode. In deoxy HbA this mode remains at 216 cm^{-1}, but it weakens and the line shape becomes asymmetric. In contrast, in NES des-Arg HbA this mode shifts to higher frequency while decreasing in intensity. The expression of quaternary structure differences in the iron-histidine bond is therefore dependent on an interaction with water. It is attractive to postulate that water molecules situated in the heme crevice interact directly with the heme or the proximal histidine in vitro. However, we cannot exclude a long-range effect in which the tertiary conformation at the heme is controlled by hydrogen-bonding interactions with water at the surface of the protein. Also, the effect of thermal contraction of the globin on the tertiary structure of the protein remains to be explored.

There have been very few reports of conformational changes induced in biomolecules on freezing. In heme proteins changes in the spin equilibrium of methemoglobins (*16*), in ligand orientation in oxycobalt myoglobin (*17*), and in intermolecular order in cytochrome c peroxidase (*18*) have been reported. Those results, coupled with our observations that freezing can alter the properties of the biologically active site in deoxyhemoglobin, demonstrate that the utmost care must be utilized in drawing functional implications from studies in which heme proteins have been probed under nonphysiological conditions. On the other hand, the recognition of these differences may allow the factors that influence biological function to be determined.

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1. Abbreviations: T, low oxygen affinity; R, high oxygen affinity; NES, *S*-(*N*-ethylsuccinimido)-cysteinyl; Arg, arginine; IHP, inositol hexaphosphate.
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The Posterior Pituitary: Regulation of Anterior Pituitary Prolactin Secretion

Abstract. *Removal of the posterior pituitary from anesthetized male rats results in a prompt and significant increase in circulating prolactin that is reversed by the injection of dopamine. Posterior pituitary extracts, which contain high concentrations of endogenous dopamine, inhibit prolactin secretion from isolated anterior pituitary cells. This inhibition is prevented by incubation of the cells with the dopamine receptor antagonist (+)-butaclamol. The data show that posterior pituitary dopamine reaches the anterior pituitary via the short hypophysial portal vessels and participates in the regulation of prolactin secretion.*

Dopamine is widely accepted as a physiological prolactin-release inhibiting hormone (PIH) for two reasons: the presence of dopamine in hypophysial portal blood at higher concentrations than in the systemic circulation and the demonstration of a reciprocal relation between hypothalamic dopamine secretion and pituitary prolactin release under a variety of endocrine states (*1*). Indeed, dopamine inhibits prolactin secretion in vitro at a range of concentrations found in hypophysial portal blood (*2*). Further supporting evidence that dopamine acts directly on the pituitary comes from the identification of specific high-affinity dopaminergic receptors in anterior pituitary homogenates (*3*).

The source of dopamine affecting the anterior pituitary is believed to be the tuberoinfundibular dopaminergic tract whose cell bodies are located in the

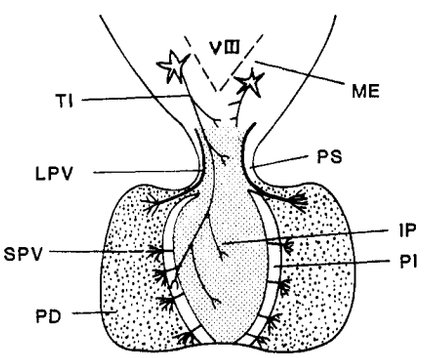

Fig. 1. Diagrammatic presentation of selected vascular and neural connections of the rat pituitary gland, shown here from a dorsal aspect. The pars distalis (*PD*) or anterior pituitary is supplied with long portal vessels (*LPV*) from the median eminence (*ME*) of the hypothalamus via the pituitary stalk (*PS*), and short portal vessels (*SPV*) from the infundibular process (*IP*) or neurohypophysis, via the pars intermedia (*PI*). The tuberoinfundibular (*TI*) dopaminergic pathway has cell bodies in the arcuate nucleus near the third ventricle (*VIII*), and nerve terminals in the median eminence as well as in the neurointermediate lobe, commonly referred to as the posterior pituitary. Redrawn and modified from Daniels and Prichard (*7*).

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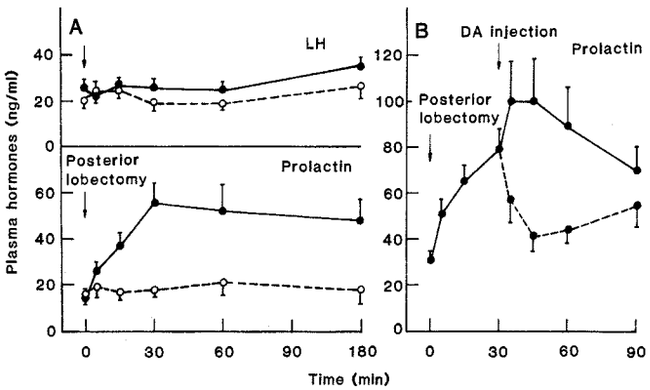

Fig. 2. (A) Effect of posterior pituitary lobectomy on plasma prolactin and LH in urethane anesthetized male rats. After exposure of the pituitary by a parapharyngeal approach, the posterior lobe was removed by a gentle suction with a modified 19-gauge needle. Blood (0.3 to 0.5 ml) was withdrawn from a cannulated femoral artery immediately before posterior lobectomy. (●) (N = 18) or sham operation (○) (N = 14) and at designated time intervals thereafter. Plasma was analyzed in duplicate for prolactin and LH by radioimmunoassays. Means ± standard error are shown. (B) Effect of injection of dopamine (*DA*) on the posterior lobectomy–induced prolactin elevation. Male rats (N = 33) were subjected to posterior lobectomy and blood was withdrawn from the femoral artery as described in (A). Thirty minutes after the lobectomy, either (solid line) dopamine (150 ng in 0.3 ml of saline containing 0.1 mM ascorbic acid, pH 7.35; N = 18) or (broken line) the solvent vehicle (N = 15) was injected into a cannulated internal carotid artery. Means ± standard error are shown.

arcuate nucleus with terminals in the median eminence as well as in the posterior pituitary (*4, 4a*) (Fig. 1).

In spite of their anatomical juxtaposition and extensive vascular interconnections, the two lobes of the pituitary are dissimilar in terms of embryonic origin, neural components, cell types, and hormonal profiles. Therefore, it is generally held that they are not functionally related. However, in view of the high concentration of dopamine in the posterior lobe (*5, 6*), the short hypophysial portal vessels connecting between the posterior and anterior lobes (*7*) (Fig. 1), and the prescribed role of dopamine as a physiological PIH, we investigated whether the posterior pituitary participates in the regulation of anterior pituitary prolactin release.

Adult Wistar male rats were anesthetized with urethane and the pituitary was exposed by the parapharyngeal approach (*8*). After drilling a hole in the basosphenoid bone, we cut the dura and removed the posterior lobe by controlled aspiration, using a 19-gauge needle with a bent tip. Controls received sham operations, the aspirating needle being inserted and withdrawn without aspiration. The procedure of posterior lobectomy takes approximately 20 to 25 minutes, causes very little bleeding, and results in no visible damage to the anterior pituitary or pituitary stalk. Blood samples (0.3 to 0.5 ml) were collected from a cannulated femoral artery immediately before posterior lobectomy and at specified time intervals thereafter; the blood was replaced with equal volumes of saline. Plasma was separated by centrifugation and analyzed for prolactin and luteinizing hormone (LH) by radioimmunoassays.

As shown in Fig. 2A, plasma prolactin increased from 14.3 ± 1.7 ng/ml (mean ± standard error) at time 0 to 55.1 ± 8.3 ng/ml 30 minutes after posterior pituitary lobectomy. Plasma prolactin in the posterior lobectomized rats remained significantly higher than that in the controls ($P < .01$) for at least 2 hours. To test whether this increase in prolactin resulted from the removal of a specific prolactin inhibiting agent, we also measured plasma LH. We found no difference in plasma LH between posterior lobectomized and control male rats for the duration of the experiment (Fig. 2A).

To determine the effect of dopamine injection on the posterior lobectomy-induced prolactin release, we subjected male rats to posterior lobectomy and withdrew blood from a femoral artery as described before. Thirty minutes after the posterior lobectomy, half of the rats were injected with dopamine (150 ng in 0.3 ml of saline containing 0.1 mM ascorbic acid, pH 7.3) via a cannulated internal carotid artery. The other half were injected with the solvent vehicle (*9*). As shown in Fig. 2B, dopamine had an immediate effect on the increased plasma prolactin, reducing it by 40 percent within 5 minutes and by 60 percent after 15 minutes to a value not significantly different from that seen before the posterior lobectomy. The effect of dopamine was short-lived, however, and 60 minutes after the injection, plasma prolactin in the dopamine- and vehicle-injected rats was not significantly different.

Primary cultures of anterior pituitary cells were used to determine if this PIH activity of the posterior pituitary could be demonstrated in vitro, and whether it was due to the presence of dopamine.

Adult rats were used as donors of anterior pituitary cells (*10*). Catecholamines in posterior pituitary extracts were determined by a double isotope radioenzymatic assay (*6*), and prolactin secreted by the cells into the medium was analyzed by radioimmunoassay.

As shown in Fig. 3, dopamine at the concentrations of $10^{-8}M$ and $10^{-7}M$ caused, respectively, a 25 and 70 percent inhibition of prolactin secretion from isolated anterior pituitary cells. Incubation of the cells with $10^{-7}M$ dopamine in the presence of the dopamine receptor antagonist (+)-butaclamol ($10^{-7}M$), prevented this inhibition, whereas butaclamol alone had no effect on prolactin release. Cell incubation with four equivalents of posterior pituitary extract (containing $3.1 \times 10^{-8}M$ dopamine and $0.8 \times 10^{-8}M$ norepinephrine) resulted in 40 percent inhibition of prolactin secretion. Incubation of the cells and extract together with butaclamol blocked this inhibition.

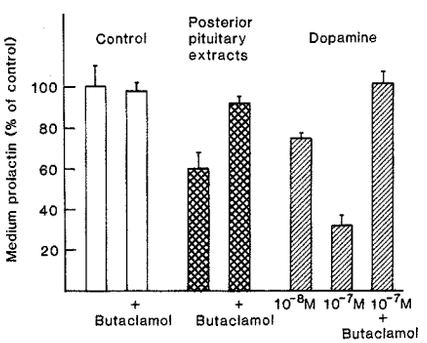

Fig. 3. Inhibition of prolactin secretion from isolated rat anterior pituitary cells by dopamine and posterior pituitary extracts and its reversal by the dopamine receptor antagonist (+)-butaclamol ($10^{-7}M$). Cells were dispersed with collagenase and hyaluronidase as described by Ben-Jonathan *et al.* (*10*), and cultured for 4 days in a modified Dulbecco medium. The cells (200,000 per milliliter) were then rinsed and incubated for 3 hours in Medium 199 containing 0.1 mM ascorbic acid (control) or the different treatments. The medium was analyzed in triplicate for prolactin by radioimmunoassay. Each value is mean ± standard error of four replicates. The extract (four posterior pituitary equivalents per treatment) was prepared as follows: a pool of rat posterior pituitaries was homogenized in 95 percent methanol and 5 percent 0.1N HCl with 1.0 mM ascorbic acid and centrifuged at 20,000g for 30 minutes; the supernatant fluid was dried under nitrogen. The residue was redissolved in Medium 199 and portions were taken for determination of prolactin by radioimmunoassay and catecholamines by the radioenzymatic assay of Ben-Jonathan and Porter (*6*). The concentration of catecholamines per treatment was $3.1 \times 10^{-8}M$ dopamine and $0.8 \times 10^{-8}M$ norepinephrine. Epinephrine was undetectable.

The data indicate that the posterior pituitary contains a substance capable of inhibiting prolactin secretion both in vivo and in vitro. Our previous study with isolated anterior pituitary cells showed that this PIH activity of the posterior lobe had a dose-dependent characteristic and that vasopressin and oxytocin were ineffective in modifying prolactin secretion (*11*). On the basis of the reversal of the posterior lobectomy-induced prolactin increase by dopamine, the abolishment of this PIH activity in vitro by butaclamol, and the endogenous concentration of dopamine, we propose that the active PIH in the posterior pituitary is dopamine.

We have recently reported (*12*) that 85 percent of estrous female rats subjected to long-term posterior lobectomy had increases in circulating prolactin sufficient to induce pseudopregnancy for 11 days. Water consumption increased four- to fivefold immediately after posterior lobectomy, indicating a severe vasopressin deficiency, but was only twice above control water consumption after 12 to 14 days. This and the fact that the increase in prolactin was not sustained, because all posterior lobectomized females resumed normal cyclicity, could be explained by the regeneration of the posterior lobe taking place during this time (*13*).

Neither the in vivo nor the in vitro methods reported here are sufficiently refined to determine the relative role of the median eminence (long portal vessels route) compared with the posterior pituitary (short portal vessels) in the dynamic regulation of prolactin secretion. Furthermore, it is questioned whether dopamine is synthesized exclusively in hypothalamic nuclei and is only stored in the posterior lobe as is the case with vasopressin and oxytocin (*14*). Some degree of independence of hypothalamic contribution is suggested by our results showing that posterior pituitaries incubated in vitro are capable of synthesis of dopamine de novo from tritiated tyrosine. Indeed, the presence of tyrosine hydroxylase and the absence of dopamine β-hydroxylase in the posterior lobe has been reported (*5*). Thus, to gain a better insight into the participation of the posterior lobe in the regulation of prolactin secretion, a correlation should be made between possible changes in dopamine concentration or turnover in the posterior lobe and alterations in prolactin secretion under various endocrine conditions.

The present study demonstrates that there is a functional dependency between the two lobes of the pituitary, at least with respect to one of the anterior pituitary hormones. In view of the high concentration of other releasing or inhibiting hormones such as thyrotropin-releasing hormone (*15*), somatostatin (*16*), corticotropin-releasing hormone activity (*17*), and the enkephalins (*18*) in the posterior lobe, other types of interactions might also occur in the pituitary.

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Centrilobular Injury Following Hypoxia in Isolated, Perfused Rat Liver

Abstract. *Hypoxia was produced in isolated, hemoglobin-free, perfused rat liver by reducing the flow rate of oxygen-carrying fluid entering the organ. The procedure caused anoxia in centrilobular regions. In these anoxic areas, structural derangements developed rapidly, characterized by bleb-like protrusions of hepatocyte plasma membrane through fenestrations in the sinusoidal endothelium. Periportal tissue remained normoxic and was completely spared. Cellular injury resulting from localized anoxia may play an important role in the pathogenesis of centrilobular liver disease.*

Centrilobular liver injury occurs as the consequence of circulatory disorders and the toxicity of various drugs (*1*). This type of injury has been postulated to reflect the fact that the central region of the liver lobule is the last to receive blood-borne oxygen and metabolites (*2*). Long-term exposure to alcohol, in particular, causes centrilobular injury in experimental animals and man (*3*). In addition, both long- and short-term exposure to alcohol causes an increase in oxygen consumption by the livers of laboratory rodents (*4*).

On the basis of these observations, Israel *et al.* (*5*) hypothesized that alcohol increases the oxygen requirement of the liver such that those regions most distant from the supply receive inadequate oxygen, become anoxic, and undergo a sequence of cellular changes leading to necrosis. The hypothesis is supported by the recent demonstration of a substantial (200 to 300 torr) intralobular oxygen gradient in isolated, hemoglobin-free, perfused rat liver (*6, 7*). In livers from rats treated with alcohol over extended periods this intralobular gradient increased 30 percent, in parallel with an increase in hepatic oxygen uptake (*7*). We have now shown that stable, circumscribed zones of anoxia develop in response to insufficient oxygen delivery and that such anoxia leads to centrilobular injury and necrosis.

Since liver function and oxygenation cannot be monitored with precision in the intact animal, we used livers isolated

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