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Prolactin Binding Sites in the Rat Brain

Abstract. *The principles of the competitive-binding assay were used in conjunction with light microscopic radioautography to demonstrate specific prolactin binding sites localized on ependyma of the rat choroid plexus, a previously unknown prolactin target tissue.*

The competitive-binding assay is routinely used to detect the presence of specific, hormone binding sites in vitro. The technique is based on the principle that radioactively labeled and unlabeled hormone compete equally for receptor sites; specific binding is defined as the difference between bound hormone in samples receiving labeled hormone alone (experimental) and those receiving labeled hormone in the presence of an excess of unlabeled hormone (control). Recently, the technique has been applied in combination with radioautography to determine the distribution of specific protein hormone binding sites in vivo (*1–3*). Utilizing the principles of competitive binding in conjunction with light microscopic radioautography, we have localized specific prolactin binding sites on ependyma of the choroid plexus in the rat brain.

Adult, sexually mature male (200 to 250 g) and female (180 to 220 g) Wistar rats were anesthetized with an intraperitoneal injection of 35 mg of chloral hydrate per 100 g of body weight. Experimental rats (two male and two female) received an intracardial injection of ovine ^{125}I-labeled prolactin (4.48×10^8 dis/min) (*4*), and control rats (two male and two female) received an identical dose of ^{125}I-labeled prolactin plus a 500-fold excess (830 μg) of unlabeled ovine prolactin. Animals were perfused through the left ventricle with Ringer's lactate and then with Bouin's fixative. All male rats and one experimental and control female were perfused 5 minutes after hormone injection, while the remaining females were perfused 15 minutes after hormone injection.

Sections (4 μm thick) embedded in paraffin were cut from corresponding brain regions of experimental and control animals and were mounted on the same slide, stained with hematoxylin and eosin, and coated with Kodak NTB2 emulsion (*5*). Radioautographs were exposed for 12 to 18 days and were then developed for 6 minutes in Kodak D-170. Additional nonradioactive brain tissue (from one male and one female) were processed in an identical manner to determine background counts and to confirm the absence of radioautographic artifacts.

Quantitative comparisons of intensities of radioautographic reactions were carried out by counting silver grains per frame at a magnification of 1600 in a Zeiss photomicroscope. Each frame represented a unit area of 6960 μm^2. Eight frames per animal were counted over the choroid plexus and 25 frames per animal over the cerebral cortex, subfornical organ, subcommissural organ, median eminence (dorsal and ventral portions), hypothalamic ventromedial nucleus (VMN), dorsomedial nucleus (DMN), arcuate nucleus, and medial preoptic-anterior hypothalamic area (MPOAH) (*6*).

The brains of males and females (both those perfused 5 minutes and those perfused 15 minutes after hormone injection) exhibited similar results in all regions examined. The data in Table 1 summarize the results.

The strongest radioautographic reaction, that is, the greatest concentration of [^{125}I]prolactin, was found over the ependyma of the choroid plexus of ex-

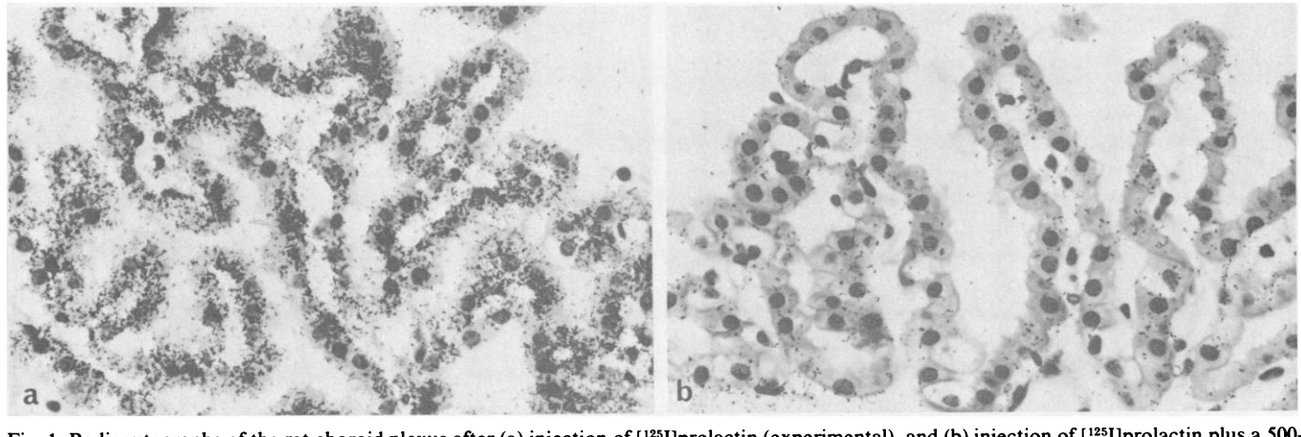

Fig. 1. Radioautographs of the rat choroid plexus after (a) injection of [^{125}I]prolactin (experimental), and (b) injection of [^{125}I]prolactin plus a 500-fold excess of unlabeled prolactin (control). A reduction of 81 percent in the number of silver grains occurs over ependyma of control tissue (×400).

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Table 1. Comparison of intensities of radioautographic reactions in various regions of the female rat brain 5 minutes after injection of ^{125}I-labeled prolactin. Data show the mean ± standard error.

Brain region	Grains per 6960 μm^2		Percentage change in radioautographic reaction
	[^{125}I]Prolactin	[^{125}I]Prolactin plus excess unlabeled prolactin	
Choroid plexus	725 ± 43	141 ± 64	−80.6*
Median eminence			
Dorsal portion	242 ± 13	287 ± 11	+18.6†
Ventral portion	209 ± 4	210 ± 5	+ 0.5
Arcuate nucleus	74 ± 5.8	83 ± 5.0	+12.2
Dorsomedial nucleus	6.6 ± 0.6	6.9 ± 0.3	+ 4.6
Ventromedial nucleus	6.0 ± 0.3	6.4 ± 0.6	+ 6.7
Medial preoptic-anterior hypothalmic area	6.7 ± 0.2	6.5 ± 0.3	− 3.0
Subfornical organ	124 ± 6.4	146 ± 4.4	+17.7†
Subcommissural organ	8.4 ± 0.4	8.2 ± 0.4	− 2.4
Cerebral cortex	7.3 ± 0.5	6.9 ± 0.5	− 5.5

*$P < .001$ (Student's t-test). †$P < .05$ (Student's t-test). Background counts, 6.3 ± 0.6.

perimental animals (Fig. 1a). The choroid plexus in control animals (Fig. 1b) exhibited an 81 percent reduction in the radioautographic reaction.

Radioautographic reactions occurred over the arcuate nucleus, median eminence, subcommissural organ, and subfornical organ of experimental and control animals (Table 1). Within the arcuate nucleus the highest concentration of silver grains was over the ventromedial portion of the nucleus. The radioautographic reaction rapidly dissipated toward the cell-poor zone between the arcuate and ventromedial nuclei. Silver grains were also scattered beneath the arcuate nucleus and followed the ventral contour of the brain to the lateral border of the arcuate nucleus. Over the subcommissural organ the radioautographic reaction was very weak, but grain counts were still statistically higher than the background counts. In the region of the arcuate nucleus, the subcommissural organ, and the ventral portion (external layer) of the median eminence, no statistical differences in grain counts were found between experimental and control animals (Table 1). In contrast, the dorsal portion of the median eminence (ependyma, subependyma, and dorsal part of the interanal layer) as well as the subfornical organ showed a statistically significant increase in the number of silver grains in control animals when compared to experimental animals (Table 1).

No radioautographic reaction was found over the MPOAH, VMN, DMN, and cerebral cortex of either experimental or control specimens (Table 1). The number of silver grains in these areas did not differ statistically from the background counts.

An 81 percent reduction in the radioautographic reaction after an excess of unlabeled hormone was injected indicates the presence of specific prolactin binding sites on ependyma of the choroid plexus, a previously unknown prolactin target tissue. Specific prolactin binding sites have also been found in vitro on the choroid plexus of sheep, rat, pig, and rabbit (*7*). Furthermore, when an identical methodology is used, the radioautographic reaction caused by [^{125}I]-insulin over choroid ependyma is not reduced in the presence of excess unlabeled insulin (*3*), indicating the reduction in radioautographic reaction with [^{125}I]prolactin is hormone-specific. Specific prolactin binding sites on the choroid plexus may reflect (i) involvement of prolactin in the regulation of cerebrospinal fluid (CSF) composition or (ii) specific receptors for an uptake and transport mechanism for prolactin from vasculature to CSF. The possible involvement of prolactin in the regulation of CSF composition may represent a major role for prolactin in males. Prolactin has been reported to alter plasma and urine electrolyte balance in rats (*8*), cats (*9*), and humans (*10*), and may fulfill a similar role in regard to the CSF.

Prolactin inhibits its own secretion from the anterior pituitary gland by way of a "short-loop" feedback circuit to the hypothalamus (*11*). Iontophoretic application of prolactin in the rat CNS (*12*) has suggested that most prolactin-responsive neurons are located in the DMN and VMN. Electrophysiologic responses in the rabbit CNS to vascular injections of prolactin (*13*) show a predominant distribution in the MPOAH. The absence of radioautographic reactions in these areas may suggest an inaccessibility of these regions to vascular prolactin as a result of the blood-brain barrier. It is possible that prolactin could be delivered to these areas over a longer time period than used in this study by a "round-about" CSF route (*14*). Compatible with this is the accessibility of most neural tissue to the CSF (*15*) and the observation that the CSF concentration of prolactin parallels the plasma concentration in rats and humans (*14*). The prolactin binding sites on the choroid plexus could play a role in the uptake of prolactin from blood into CSF.

The median eminence and subfornical organ lack a blood-brain barrier (*16*); the arcuate nucleus shares in this deficiency because of its close proximity to the highly vascular and permeable median eminence. The radioautographic reactions in these regions would in part reflect their accessibility to vascular prolactin and may represent nonspecific fixation of hormone or pinocytosis by glia and ependyma. In particular, pinocytosis by glia and ependyma at vastly supraphysiologic concentrations of prolactin may explain the increase in silver grains over the subfornical organ and dorsal portion of the median eminence in control specimens (*2*).

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Nonhuman Models of Hereditary Porphyrias

While bovine protoporphyria may prove to be a more useful subject of clinical studies than any one of the numerous alternatives, it is not "the first known animal model for any of the hereditary human porphyrias other than so-called 'erythropoietic' porphyria" (*1*).

High levels of free porphyrins in living tissues occur in at least four phyla of multicellular animals in addition to the Chordata (*2*). A free porphyrin now known to be protoporphyrin IX was found in the annelid body wall as early as 1886, by the British zoologist MacMunn (*3*). In the 20th century, the wide zoological distribution of free porphyrins and the great structural diversity of naturally occurring compounds has been investigated extensively (*4*). Among the adults of a species the compounds are essentially ubiquitous, leaving little doubt of their hereditary basis. At least in the annelids and the asteroid echinoderms, exposure to daylight is often accompanied by the classical clinical symptoms of the human porphyrias, for example, cutaneous lesions, edema, and, eventually, death.

The interest in these facts is less the correction of a minor historical oversight and more the ecological and evolutionary aspects of the distribution of porphyrias. In phyla such as the echinoderms and nemertines, porphyrias (including protoporphyria) are found in species that do not have protoheme proteins in the blood or in nerve and muscle tissues (*2*). Thus there are nonhuman models that clearly cannot be associated with erythropoiesis, or related forms of protoheme synthesis. In the annelids, however, many of the porphyrias (also including protoporphyria) are associated with the biosynthesis of oxygen carriers in the blood, either of two high-molecular-weight extracellular heme proteins (*5*). Porphyrias have not been found in species with only intracellular blood oxygen carriers, suggesting an inherent advantage of the red cell environment in the control of the biosynthetic pathway (*6*).

Moreover, porphyrias are found only in annelid species or in parts of their bodies that do not normally encounter daylight; they do not seem to occur among the errant families or in respiratory and feeding organs, which are protruded from the darkness of the tube (*5*). Thus the inheritance of the condition seems to be highly responsive to selection pressures of the environment.

In the higher animal phyla, nonhuman porphyrias are either nonexistent, as in the arthropods and urochordates, or rare, as in the various classes of chordates (*2*). The phylogeny suggests a relatively recent origin of the metabolic control mechanism that render the condition a rare disease and not the predominant condition in many species.

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Because we are very conscious of the normal accumulation of large amounts of porphyrin in several phyla of multicellular animals (as well as in unicellular organisms, root nodules, and the like), we carefully limited our model comparison with "hereditary human porphyrias" (*1*). The latter have been classified and defined in terms of specific clinical, anatomical, and biochemical manifestations (*2*) which distinguish them from the examples cited by Mangum (*3*). Nonetheless, in a recent report to the NIH, we raised a similar issue, broadening our earlier concepts of hereditary porphyria. Thus, we have now classified the well-known occurrence of protoporphyrin in brown eggshells and uteri of Rhode Island Reds, Japanese quail, and other hens as examples of hereditary uterine porphyria. Similarly, several rodent species exhibit Harderian gland porphyria. A transitory period of normal "physiological" porphyria has been reported in fetuses of many species. Numerous other examples might be cited. We agree that use of the broad term "hereditary porphyria" should be as broad as is its natural normal occurrence, being defined more specifically in each instance in terms of its unique features. The challenge, then, is to determine the special rate-limiting biosynthetic reactions that lead to the porphyrin accumulation and to their possible biochemical and physiological implications, some of which are considered by Mangum.

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