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Review

The prolactin releasing peptides: RF-amide peptides

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Abstract. Although dopamine is considered the major hypothalamic controller of prolactin release from the anterior pituitary gland, there is evidence that a yet to be discovered prolactin releasing factor (PRF) also exists in brain. Recently, two peptides were isolated, products of the same prohormone, that were reported to have significant prolactin-releasing activity. These peptides, called prolactin releasing peptides, are not accepted by all investigators to be in fact PRFs. Instead, it appears that their widespread distribution in brain and the presence of receptors for the peptides in sites unrelated to neuroendocrine function are the basis for a variety of central nervous system action including activation of the autonomic nervous system. Thus, these peptides may not be PRFs, but instead neuroactive agents that are involved in many brain circuits with divergent functions.

Key words. Prolactin; releasing factors; autonomic function; brain.

Introduction

Dopamine (DA), of hypothalamic origin, is recognized to be the major, physiologic regulator of prolactin (PRL) secretion from the anterior pituitary gland [1–3]. The inhibitory effect of DA, released into the vicinity of the fenestrated capillary endothelium of the hypophysial portal vessels in the median eminence, is best appreciated when either DA production is blocked or the portal vessels themselves are severed [1, 2]. Indeed, relative DA absence or insensitivity accounts for a major portion of the observed cases of hyperprolactinemia, a condition that in menstruant-aged women leads frequently to amenorrhea and infertility [2]. Fortunately, many of those women respond to pharmacologic intervention with DA mimetics [2] and in fact many cases of hyperprolactinemia are controlled by drug therapy. However, a significant portion of the patient population displaying hyperprolactinemia are DA resistant, suggesting either the absence of the normal dopaminergic inhibitory tone because of a relative insensitivity to the catecholamine, or the presence of a factor that drives the lactotroph to produce and secrete abnormally high levels of hormone.

Evidence for the production of PRL-releasing factors (PRFs) in hypothalamus is abundant, and yet exhaustive attempts to purify and identify novel PRFs have failed [3–7]. Just the same, there appear to be stimulatory factors of hypothalamic origin and the existence of a DA-resistant subpopulation of hyperprolactinemic patients justifies, indeed drives, the search for additional neuroendocrine factors that might control the pituitary production and secretion of PRL. The search for the missing, physiologic PRF has uncovered the PRF activity of several previously identified peptides and led to the identification of at least one new hypothalamic peptide [7–10].

Known peptides with PRF activity

Knowledge of the integrative physiology of lactation led several investigators to hypothesize that oxytocin (OT) re-

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leased in response to suckling, not only stimulated milk ejection in the breast [11], but also, because of its close proximity to the anterior pituitary gland when released into the sinusoids of the posterior pituitary gland, played a neuroendocrine role in the subsequent stimulation of PRL release from the lactotroph [12]. In fact, the release of OT from the posterior pituitary gland precedes PRL secretion during lactation and steroid-induced hormone release [13], and OT receptors are present in the anterior lobe [11]. The presence of OT in hypophysial portal blood [14] and the apparent physiologic regulation of those blood hormone levels gave support to a proposed PRF activity for OT. We were able, in 1983, to provide evidence for a direct, dose-related action of OT on the lactotroph [12], and later to demonstrate the first evidence for a physiologic role for endogenous OT in the regulation of PRL secretion [13]. However, neither antagonists nor antibodies directed against endogenous OT could completely abrogate the PRL surge to steroid priming or lactation, which led us to speculate that other peptides of hypothalamic origin acted to oppose the inhibitory effect of DA [7, 13].

It was at that time already known that another hypothalamic peptide, thyrotropin-releasing hormone (TRH), which was recognized to be the major neuroendocrine factor controlling thyrotropin (TSH) release, also acted pharmacologically to stimulate PRL release both in vitro [15] and in vivo [1]. Although some investigators believe that TRH may play a physiologically relevant role in the neuroendocrine regulation of PRL secretion [16], others do not agree [see 2, 3]. Indeed, the fact that TSH and PRL releases are not always simultaneous or at least coordinated in vivo argues against the importance of the PRL-releasing action of TRH, at least under normal physiologic conditions [2]. All the same, TRH is a valuable tool for clinical testing of lactotroph function [2].

Another hypothalamic peptide that was demonstrated virtually simultaneously by three independent groups to stimulate PRL release in vitro [17–20] was vasoactive intestinal polypeptide (VIP). We had demonstrated previously the presence of VIP in median eminence and hypophysial portal blood [21] predicting our [18] and other [19, 20] demonstrations of the PRF activity of the peptide. However, as would later be the case for OT [7, 9, 13], blockade of VIP's action with selective antagonists or compromise of its function with specific antibodies failed to completely nullify the PRL responses to stress, lactation or steroid priming [22–24].

Numerous other known peptides were reported to stimulate PRL (fig. 1) release in vitro, under selective conditions, but none were reproducibly as potent as OT, TRH or VIP, and those peptides could not account for the non-OTergic, non-VIPergic and non-TRHergic PRF being purified from hypothalamic extracts [5, 6, 9]. Those PRFs were peptidergic in nature, but their identity remained for many years elusive to standard peptide purification approaches [6]. In fact, the search for the missing peptidergic PRF stagnated for several years, as many investigators sought more fundable research foci.

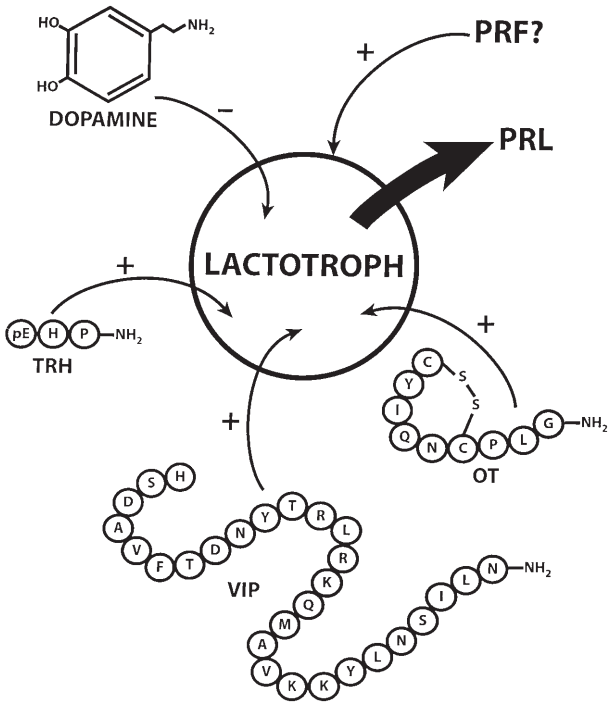

Figure 1. Hypothesized neuroendocrine mechanisms controlling prolactin (PRL) release from the anterior pituitary gland. Dopamine (DA) is major regulator of hormone secretion although physiologically relevant stimulatory actions of thyrotropin releasing hormone (TRH), oxytocin (OT) and vascoactive intestinal peptide (VIP) have been demonstrated. It is believed that a physiologically relevant, but as yet unidentified, prolactin releasing factor (PRF) exists in brain.

Novel methodology reenergizes the hunt for the missing PRF

The advent of modern molecular technologies heralded a renaissance in the study of neuroendocrine regulation of anterior pituitary function. Investigators able to accurately monitor the regulation of the production and processing of known neuropeptides in the hypothalamus and numerous novel signaling systems were identified and an ever-expanding catalog of 'orphan' receptors developed. The proteins, classified as receptor proteins because of their structural homology to known, seven transmembrane-spanning-domain molecules recognized to be receptors for such diverse ligands as catecholamines and in some cases, hypothalamic neuropeptides [25], were identified by cloning techniques. Many lacked identified biologic ligands. One of these, UHR-1 was originally identified in hypothalamic extracts by subtraction cloning and in fact named because of its presence in the hypothalamic

'Zeitgeber', the suprachiasmatic nucleus [26]. The discoverers of this orphan receptor originally thought that it might transduce signals important in the regulation of circadian rhythms.

The ligand for UHR-1, and the human counterpart hGR3 [27], remained unknown until scientists at Takeda Chemical Industries employed it in high-throughput screening assays to identify a new class of hypothalamic neuropeptides. Not only did they identify the peptide of hypothalamic origin that bound the orphan receptor, but they also recognized that one of the highest densities of UHR-1 expression was the anterior pituitary gland. Peptidergic fractions of hypothalamus stimulated arachidonic acid metabolism in CHO cells transfected with the hGR3 receptor, and this reporter assay (arachidonic acid metabolite release) was employed to obtain partial sequence identity of the bioactive ligands purified. Complementary DNAs (cDNAs) were then isolated and determined to encode a preprohormone that varied in length depending upon species from 98 (bovine), to 87 (human) to 83 (rat) amino acids in length. More recently, Langmead and colleagues [28] asserted that the human PrRP receptor identified by Hinuma [27], hGR3, is essentially identical to the G-protein-coupled receptor originally identified as GPR-10 [29]. In HEK293 cells transfected with GPR-10, both rat and human PrRP bind with high affinity [28].

The preprohormone contained a typical N-terminal signal peptide and two internal cleavage sites that predicted post-translational processing to result in the formation of potentially two peptides, one of 31 and another of 20 amino acids, constituting the C-terminus of the larger peptide (fig. 2). Highest expression of the messenger RNA (mRNA) encoding these peptides was observed in medulla oblongata, with some mRNA detected in hypothalamus, where the highest peptide bioactivity was localized. These peptides were named prolactin releasing peptides (PrRP-31 and PrRP-20) because of their ability to stimulate PRL secretion from a rat pituitary adenoma-derived cell line (RC-4B/C cells) and from primary cultures of anterior pituitary cells harvested from lactating female rats, a model recognized by most in the field to be the most sensitive to the PRF activity of a variety of hypothalamic peptides [3, 18]. These authors reported the potency of PrRP-31 in RC-4B/C cells to equal that of TRH. The PRL-releasing activity in cells harvested from lactating female rats appeared selective since the authors reported no significant effects of PrRP-31 on luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, growth hormone or adrenocorticotropin release under basal conditions. Furthermore, the PRF activity of PrRP-31 was expressed in cells that did not respond to a variety of other hypothalamic peptides reported by others to stimulate PRL release in vitro, including VIP, oxytocin and galanin. We found this perplexing, since in our hands VIP [18] and OT [12, 13] are potent releasers of PRL in cells harvested from lactating female rats.

Our group was excited that the missing PRF might finally have been identified, and immediately obtained peptide to further characterize the reported PRF activity of PrRP-31 and PrRP-20. To our disappointment [30], neither peptide was active in cells harvested from male rat pituitary donors, and both were only active in cells harvested from random cycle female rats at very high doses (100–1000 nM). Similarly, in our hands cells harvested from lactating female rats were also relatively insensitive to the PRF activity of PrRP-31. Although the affinity of rat PrRP for the UHR-1 receptor has been identified [31], the doses required in vitro for PRF activity are certainly in excess of the IC_{50} (the dose required to displace 50% of labeled peptide bound to the receptor) determined in those binding studies (micromolar versus nanomolar) and well in excess of doses (nanomolar) required for the PRF activity of TRH, OT or VIP in cells similarly harvested and cultured [3, 13, 18]. Furthermore, plasma levels of PrRP in female rats have been reported to be 0.14 fmol/ml [32]. Thus, whereas levels of PrRP in female rat plasma are approximately 5.0 pg/ml, doses of at least 350 ng/ml were required to observe PRF activity in vitro. Affinities described for the human PrRP receptor, GPR-10/hGR3, transfected into HEK293 cells were reported to be in the mid to high picomolar range, but no data are

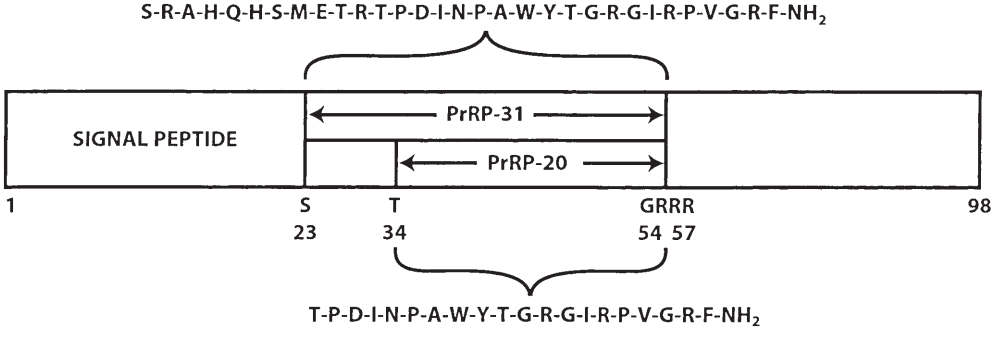

Figure 2. Schematic structure of the rat prolactin-releasing-peptide prohormone, indicating the location of the two PrRPs and their amino acid sequences (redrawn from [54]).

available describing binding affinities in normal human tissue or in rat pituitary gland, which is the current in vitro model for PRL release.

We felt that it was possible that PrRP exerted physiologically relevant PRF actions during the initial phase of hormone secretion when DA levels in portal plasma fell (i.e. DA withdrawal), and this would not have been observed in first studies when cells were incubated in the absence of DA. Therefore, we conducted cell culture protocols similar to those described by Martinez-Escalera [33] in which cells cultured in the presence of physiologic levels of DA were examined for a PRL response to PrRP following a transient removal of DA-ergic tone. Whereas TRH exposure following DA withdrawal led to the expected accentuation of the PRF activity of the peptide, PrRP did not stimulate significant release of PRL either in the presence of DA or following transient withdrawal of the catecholamine (manuscript in preparation). Thus, we cannot support the contention of Hinuma and co-workers [27] that the peptide they discovered is a physiologically relevant prolactin-releasing peptide, and in our minds, the identity of the long-pursued peptidergic PRF of hypothalamic origin remains elusive.

Additional evidence against the neuroendocrine status of the PrRPs comes from peptide localization studies that failed [34–41] to detect significant quantities of peptide immunoreactivity in the external lamina of the median eminence. This is the site where recognized releasing and inhibiting factors of hypothalamic origin gain access to the hypophysial portal vasculature and the anterior lobe. Instead, PrRP immunoreactive neurons in medulla have been identified by retrograde labeling to project to the paraventricular nucleus of the hypothalamus [40]. Furthermore, the number of PrRP neurons does not differ in the medulla of brains from male, nonpregnant female and pregnant female rats [40], and the distribution of PrRP immunoreactive fibers does not change during the estrous cycle [31]. Finally, Lawrence and co-workers [42] have reported that expression of PrRP apparently decreases in lactation and fasting, suggesting a role for the peptide related to energy homeostasis and not the neuroendocrine regulation of PRL secretion.

The Takeda group [43] has reported that intravenous administration of relatively high doses of PrRP-31 (approaching 40 µg per injection) significantly stimulated PRL release in conscious rats during proestrus, estrus and metestrus. An even higher dose ([500 nmol/kg intravenous (iv), or almost 400 µg]) was required to observe a stimulatory effect of PrRP-31 in male rats. Another group [44] reported that slightly lower doses of PrRP-31, 10–15 µg iv, stimulated PRL release from anesthetized female rats during estrus. In ovariectomized, estrogen-primed rats PrRP-31 at similar doses stimulated PRL release, but not to the extent observed following similar injection of TRH. These doses of PrRP can only be viewed as supraphysiologic, since circulating levels of the peptide have been reported to be 0.13 fmol/ml or fractions of a picogram per milliliter of plasma [32]. Indeed, Jarry and co-workers [45] using lower doses of PrRP-31 were unable to demonstrate a PRF activity of the peptide when administered intravenously, although TRH was active. It should be noted that the dose of PrRP-31 in the Jarry study [45], 1 nmol, would certainly have raised plasma PRL levels well above values [32] reported to be present in rat plasma.

If not PRFs, what do the PrRPs do?

The excitement over the potential for significant biological activity of the PrRPs led to an initial wave of reports on the central nervous system localization of PrRP immunoreactivity and binding sites. There is general agreement that three main populations of PrRP-producing neurons exist with the major number found in the nucleus tractus solitarius and the reticular formation of the ventrolateral medulla. Scattered neurons also are present in the caudal aspect of the dorsomedial nucleus of the hypothalamus [34–40]. Immunoreactive fibers positive for PrRP project to paraventricular nucleus of hypothalamus (PVN), particularly the parvocellular elements, and to other rostral sites, including supraoptic nucleus, paratenial thalamus, basolateral amygdala, and the bed nucleus of the stria terminalis (BNST). Only scant fibers project to the basal hypothalamus, including the median eminence, and little staining was observed in the posterior pituitary gland. Several groups have identified the medullary cells expressing PrRP also to be tyrosine hydroxylase positive, for example the A2 and A1 noradrenergic cell groups [34, 35, 40], suggesting cosecretion of peptide and catecholamine within the brain.

The projection fibers staining positive for PrRP terminate in brain regions that express abundant mRNA for the PrRP receptor [34, 46]. In a remarkably comprehensive study, Roland and co-workers [30] observed receptor mRNA in highest intensity in the paraventricular nucleus of the hypothalamus, predominantly at its midportion adjacent to the third ventricle, a site associated less with regulation of OT and vasopressin (AVP) release and more with the role of the nucleus in stress and cardiovascular regulation [47]. Additional intense hybridization was detected in the thalamic reticular nucleus, the area postrema and the commissural aspect of the nucleus tractus solitarius, supporting a role for PrRP in central control of autonomic function. Localization of receptor message in the dorsomedial nucleus of the hypothalamus suggests a role for the peptide in neuroendocrine function or metabolic regulation [45]. Such a role is suggested by the observation that centrally administered PrRP (4 nmol) significantly inhibited spontaneous and restriction-stimulated food intake [42]. Addi-

tional potential roles for PrRP were suggested by identification of moderate intensity of message for the receptor in the medial preoptic area and nucleus (neuroendocrine control of gonadotropin secretion), the anterior parvicellular region of the PVN (autonomic/stress regulation and possible regulation of OT and AVP release), ventrolateral hypothalamus (behavioral or appetitive actions) and locus coeruleus (autonomic regulation).

We were drawn to the extrahypothalamic distribution of PrRP-immunoreactive axonal projections that matched peptide binding sites in areas of brain more related to cardiovascular function than to neuroendocrinology [47, 48]. When PrRP was injected (fig. 3) into the lateral cerebroventricle in conscious, unrestrained rats, significant elevations in mean arterial blood pressure were observed [49]. The delay in onset of this activation of the sympathetic nervous system suggests an action of the peptide distant from the site of injection, but still fairly close to the walls of the third and fourth ventricles. We would hypothesize that exogenously administered PrRP accesses receptors in the periventricular tissues such as the paraventricular nucleus of the hypothalamus, the area postrema or the commissural nucleus tractus solitarius, all areas known to be important in central autonomic regulation [48]. Site-specific injections into those areas will certainly elucidate these possibilities.

Diencephalic sites expressing PrRP receptor mRNA include areas involved in neuroendocrine regulation of anterior pituitary function [34]. Thus it was important to administer exogenous PrRP into the cerebroventricular system of conscious rats to determine if the peptide might alter the release of known hypothalamic releasing and inhibiting factors into the hypophysial portal vessels for delivery to the anterior lobe. Indeed the presence of PrRP-positive nerve terminals and PrRP receptors in parvocellular PVN predicted an action of the peptide on the release of corticotropin-releasing hormone (CRH). When administered into the cerebrospinal fluid, PrRP induced c-*fos* expression in PVN, and synaptic innervation of CRH-containing neurons by PrRP-positive terminals has been described [50]. Caution should be exercised in the interpretation of these data because the dose of PrRP employed, approximately 70 µg per injection, was without a doubt well in excess of total brain PrRP content. Central administration of PrRP, again in a relatively high dose of approximately 35 µg per injection, resulted in a significant elevation of circulating levels of adrenocorticotropin (ACTH), and this effect of PrRP could be blocked by pretreatment peripherally with the CRH antagonist, α-helical CRH [50]. Thus a neuromodulatory role for PrRP on cells producing hypothalamic releasing factors was suggested, but a lower dose of PrRP, approximately 3.5 µg, was ineffective, and no dose relationship demonstrated. For these observations to support a potential physiologic effect of PrRP in brain, dose-related stimulation should be observed and then particularly with more physiologically relevant doses of the peptide.

The presence of PrRP receptors in the rostral and mediobasal hypothalamus suggested a role for the peptide in the regulation of gonadotropin release, and certainly those distributions and the PVN localization hinted at a possible neuromodulatory role related to PRL secretion. However, in our hands no significant effects of central administration of PrRP-31 on plasma PRL or growth hormone (GH) levels could be detected (table 1) when doses of 3.5 and 10.5 µg per injection were employed. Similarly

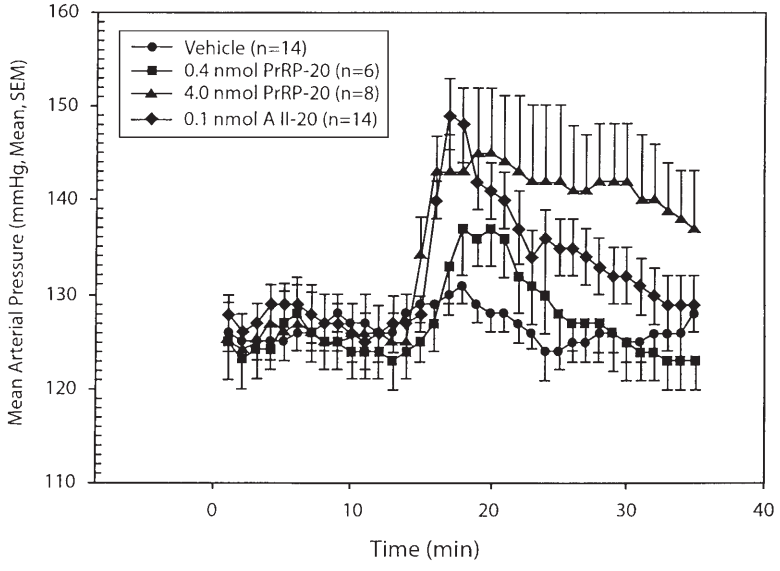

Figure 3. Intracerebroventricular administration of PrRP-20 elevates mean arterial blood pressure in conscious, freely moving, male rats. Responses are compared with those elicited by angiotensin II (A II). Reprinted with the permission of Elsevier Publishers from the original publication by Samson et al. [49].

Table 1. Failure of lateral cerebroventricular administration of PrRP-31 to significantly alter the in vivo release of PRL or GH in conscious, unrestrained male rats* (250–300 g, Sprague Dawley, Harlan, IN).

Treatment group	Time following i.c.v. injection (min)				
	0	5	15	30	60
Plasma prolactin levels (ng PRL/ml plasma, mean, SEM)					
Saline vehicle (n = 7)	23.5 ± 2.8	25.3 ± 3.0	21.2 ± 5.4	15.7 ± 3.2	13.6 ± 2.4
1.0 nmol PrRP (n = 7)	21.7 ± 4.3	23.0 ± 4.3	15.8 ± 4.7	13.4 ± 3.3	16.8 ± 2.7
3.0 nmol PrRP (n = 9)	22.7 ± 2.2	25.0 ± 2.5	19.8 ± 2.7	16.5 ± 4.1	16.8 ± 2.2
Plasma growth hormone levels (ng GH/ml plasma, mean, SEM)					
Saline vehicle (n = 7)	8.2 ± 3.1	5.8 ± 1.5	4.5 ± 1.0	12.6 ± 4.9	11.9 ± 5.3
1 nmol PrRP (n = 7)	9.1 ± 3.6	3.9 ± 0.6	4.7 ± 1.7	7.0 ± 3.1	13.6 ± 3.6
3 nmol PrRP (n = 9)	15.2 ± 4.0	6.8 ± 2.0	4.4 ± 1.0	10.2 ± 3.0	13.2 ± 3.0

* A chronic indwelling lateral cerebroventricle cannula was implanted 7 days before experimentation, and an indwelling jugular cannula implanted on the day before experimentation, as previously described [11, 12]. Within and between groups ANOVA (analysis of variance) failed to reveal any significant effects.

Seal and co-workers [51] did not observe any significant effects of centrally administered PrRP on PRL levels in peripheral plasma, although only one dose was tested (17.5 µg). Finally, Watanobe and co-workers [52] failed to observe any significant effects on PRL secretion, induced by stress or ether inhalation, when antiserum to PrRP was administered into the cerebrospinal fluid. In the study by Seal and colleagues [51], the effect of PrRP on gonadotropin release was apparently mediated by stimulatory effects of PrRP on LHRH release in median eminence. The specificity of this final observation must be demonstrated in future experiments since not only LHRH, but also galanin and VIP release was also stimulated, and no dose-response testing reported [51].

Although the distribution of PrRP receptors in the PVN might favor a role for the peptide in the regulation of CRH release and/or autonomic function, receptors are present in the PVN and supraoptic nucleus (SON), where effects on AVP and OT release could be hypothesized [34]. In fact, in conscious female rats, PrRP injection significantly elevated plasma levels of both OT and AVP, whereas in males only OT levels increased [53]. Thus actions on magnocellular elements of the PVN and SON may have physiologic relevance, and they deserve further study. However, again caution must be observed in the interpretation of these data since the effects on OT and AVP release were not demonstrated to be dose related and were observed only at a very high dose of peptide (35 µg) injected into the ventricular system [53].

Are the actions of the PrRPs limited only to the central nervous system?

Clearly, there remains controversy over the reported, direct pituitary actions of PrRP. Although the initial report of the discovery of the peptides demonstrated significant PRF activity in cells harvested from lactating female rats [27], and intravenous administration of peptide results, at least in some hands [43, 44], but not others' [45], in the release of PRL under certain physiologic states, the direct pituitary action of PrRP has not been observed by all investigators [30]. A very strong argument for a direct pituitary site of action of the peptide comes from the positive results obtained in peripheral administration studies; however, those effects on PRL release may not reflect a direct action in the pituitary gland, but instead release of the hormone secondary to a primary action in another tissue. We have observed that in addition to its hypertensive effect when administered into the ventricular system, peripheral infusion of PrRP results in rapid, dose-related elevation in mean arterial pressure [unpublished]. Because abundant receptors for PrRP are present in area postrema, a region lacking the blood-brain barrier, it is possible that peripherally administered peptide directly alters neuronal activity in this region. Alternatively, changes in baroreceptor activity secondary to the hypertensive action in the vasculature itself may affect neuronal activity in brain stem cardiovascular centers. In either case, these changes in medullary neuronal activity may, via efferent fiber pathways to the hypothalamus and brain stem reticular activating systems, result in PRL and ACTH releases secondary to the perceived stress or physiologic challenge of hypertension. Clearly, much work is needed to unravel the true meaning of the reported hypothalamic actions of the PrRPs.

Although most of the research focus has been directed to brain and pituitary actions of the peptides, Roland and coworkers [34] demonstrated convincingly that PrRP peptide and receptors are present in peripheral tissues. Satoh [31] has demonstrated PrRP binding sites in rat heart, soleus muscle, adipose tissue, kidney, adrenal gland, testes and small intestine. Roland's group went further in describing expression of the PrRP receptor messenger RNA (mRNA) in adrenal medulla, although no mRNA for the

peptide was detected [30]. It is possible that the peptide content of adrenal gland reported by Matsumoto [40] reflected peptide bound to the receptor. This peptide may have originated in plasma (source of production unknown) or it may have been delivered by the nerves innervating the chromaffin cells of the adrenal medulla. In any case a detailed examination of the possible effects of PrRP on medullary catecholamine release is now justified. Finally, Roland also described the presence of PrRP mRNA in the lamina propria of the large and small intestines [34] and although no data were shown, Hinuma and co-workers [54] have reported in preliminary in vivo studies that administration of PrRP 'influences' insulin secretion. Thus, PrRP of gut origin may play a role similar to that of gastric inhibitory peptide (GIP) in the preparation of the β cell for the appearance of glucose in portal blood subsequent to ingestion of carbohydrates.

Other RF-amide peptides

The PrRPs belong to a class of neuropeptides containing a carboxy-terminal RF-amide sequence, other members of which include neuropeptide FF (NPFF) and neuropeptide AF (NPAF), both of which may interact with brain opiate systems [55] and may modulate PRL secretion [56]. Because nonmammalian species express multiple RF-amide peptides, the Takeda group [57] searched the GenBank/EMBL databases for potential, additional RF-amide peptides in mammals. Their selection criteria included the RFGR motif, in which the final R (arginine) could serve as a cleavage site, and the C-terminal G (glycine) would serve as an amide donor. They also selected sequences with the RFGR motif that followed an upstream, signal peptide sequence. Their search resulted in the identification of two expressed sequence tags (ESTs) that could potentially express novel RF-amide peptides. cDNAs were then isolated and the peptides encoded identified. In the end three novel RF-amide peptides were predicted, and the synthetic peptides corresponding to those sequences were screened in a battery of orphan-receptor-expressing CHO cell lines. Two of the novel peptides, the RF-amide related peptides (RFRP-1 and RFRP-3), stimulated extracellular acidification in CHO cells expressing the seven transmembrane-spanning-domain receptor OT7T022, an orphan receptor previously isolated by those investigators from rat brainstem RNA by reverse-transcriptase polymerase chain reaction (RT-PCR) [57].

In an impressive series of experiments [57], Hinuma and co-workers then identified the sites in brain where these peptides, posttranslational products of the same gene, are expressed, and remarkably mapped the sites of OT7T022 receptor expression. Peptide expression is limited to a discrete region of the caudal hypothalamus, an area located roughly between the dorsomedial and ventromedial hypothalamic nuclei extending laterally from the periventricular into the lateral hypothalamic area. The only other sites of significant, albeit much lower, expression of the peptides are the eye and the testis. Receptors are expressed predominantly in hypothalamus, although within the brain receptor expression was also detected in the cerebral cortex, striatum, hippocampus, thalamus, midbrain, cerebellum, medulla and spinal cord. Receptor mRNA was also localized to optic nerve and eye, as well as pituitary gland, testis, ovary and placenta.

Within the hypothalamus, strongest expression of the receptor was detected in the PVN and the periventricular area (PeVN). These authors speculated that since some of the PeVN neurons also express tyrosine hydroxylase and therefore might be dopaminergic cells that project to the median eminence [57], the RFRPs might alter DA turnover, contributing to the hypothalamic regulation of PRL secretion. The flaw in their logic was the fact that the major dopaminergic innervation of the median eminence comes not from the PeVN, but instead from the tubero-infundibular dopaminergic neurons [1–3] of the arcuate nucleus (ARC), where the OT7T022 receptor was reported not to be expressed. In fact, thyrotropin releasing hormone TRH-expressing neurons are present in the PeVN, and the action of the RFRPs may instead be on those neuroendocrine neurons. Unfortunately, the significance of the OT7T022 receptors in the PVN was not addressed [57], and indeed, the RFRPs might act there to alter the activity of oxytocinergic neurons, cells reported to play a significant role in the hypothalamic regulation of PRL secretion [13]. Still these authors hypothesized that because of their potential innervation of dopaminergic cells of the PeVN, the RFRPs might regulate PRL secretion. When a very high dose of RFRP (10 nmol, or approximately 15 µg of peptide) was injected into the cerebrospinal fluid of conscious rats, plasma PRL levels rose significantly. Dose-dependent increases were not, as the authors asserted, demonstrated since the 1-nmol dose did not significantly elevate plasma PRL levels, and the levels of PRL in plasma in animals administered the 1-nmol dose of RFRP were not reported to differ from those in animals exposed to 10 nmol of RFRP. Data were not presented for plasma levels of other anterior lobe hormones, but the effect on PRL secretion was reported to be selective.

Since OT7T022 receptor expression was reported in pituitary gland, and since the homologous RF-amide peptide PrRP acts in the gland at high doses to stimulate PRL release, Hinuma and co-workers examined the possibility that the RFRPs might also stimulate PRL release by a direct pituitary action. Although no data were presented, the authors asserted that the RFRPs did not significantly stimulate PRL release from cultured rat anterior pituitary cells [54]. Their final conclusion [57] was that the RFRPs and PrRP 'influence secretion of PRL by different mechanisms.' Clearly, this remains to be established.

Summary and perspectives

In less than 2 years since their discovery, much has been learned about the PrRPs, peptides originally thought to be neuroendocrine factors regulating PRL release from the anterior pituitary gland. These peptides have multiple biologic activities, many predicted by the excellent localization studies that mapped the sites of their production and, importantly, the receptive fields for the axon terminals containing the peptide. There remains significant controversy over the physiological relevance of the first bioactivity described for the peptides, and a clear indication of the potential neuromodulatory effects of the peptides, independent from their reported pituitary site of action, has emerged. Little is known, however, of the physiological relevance of any of the brain actions of PrRP described to date, and as well, the mechanisms controlling peptide production or release in brain remain largely unclear. The initial findings, largely published in brief commentary or rapid communication formats, must now be corroborated by comprehensive studies using more physiologically relevant doses of the peptide with complete dose-response testing. Until selective antagonists, or genetically engineered animal models, become available, the majority of the literature on these peptides will continue to be descriptive in nature. Just the same, it is now clear that the nomenclature chosen (i.e. prolactin releasing peptides) may have been premature, since the peptides are effective in brain at lower doses than those required to express the releasing factor activity in pituitary gland, and even that action remains challenged by negative data in the literature. This does not however, diminish, the significance of the discovery of the peptides, an effort that will certainly serve not only as a model technical approach [50] for the identification of novel peptides but also the foundation of what will surely become an ever-growing literature on the biological activities of these peptides.

Future studies must examine the potential biological effects of the RFRPs in all tissues where the OT7T022 receptor is expressed, and caution should be exercised so that sweeping conclusions like those already advanced do not become interpreted in the literature as statements of fact. There is no doubt that the Takeda group has provided the scientific community with a framework for much discovery and healthy debate; however, the singular focus on PRL secretion may have been misleading and, indeed, is not yet justified by the existing scientific evidence. As in all things, discovery breeds controversy; but diligent, cautious investigation delivers fact. Hopefully, the scientific community will not consider the further study of the physiological relevance of PrRP or the RFRPs to be redundant based on the limited published literature. Instead, fertile soil remains untilled for molecular biologists, cell physiologists and whole-animal biologists in which carefully sown seeds of discovery will certainly lead to abundant harvests of relevant findings on the functions of the RF-amide peptides.

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1 Lamberts S. W. and Macleod R. M. (1990) Regulation of prolactin secretion at the level of the lactotroph. Physiol. Rev. **70:** 279–318
2 Reichlin S. (1998) Neuroendocrinology, In: Williams Textbook of Endocrinology, 9th ed., pp. 165–248, Wilson J. D., Foster D. W., Kronenberg H. M., Larsen P. R. (eds), Saunders, Philadelphia
3 Ben-Jonathan N., Arbogast L. A. and Hyde J. F. (1989) Neuroendocrine regulation of prolactin release. Prog. Neurobiol. **33:** 399–347
4 Ben-Jonathan N. (1990) Prolactin releasing and inhibiting factors in the posterior pituitary. Neuroendocrine Perspectives **8:** 1–13
5 Hyde J. F. and Ben-Jonathan N. (1989) The posterior pituitary contains a potent prolactin releasing factor: in vivo studies. Endocrinology **125:** 736–741
6 Laudon M., Grossman D. A. and Ben-Jonathan N. (1990) Prolactin releasing factor: cellular origin in the intermediate lobe of the pituitary. Endocrinology **126:** 3185–3192
7 Mogg R. J. and Samson W. K. (1990) Interactions of dopaminergic and peptidergic factors in the control of prolactin release. Endocrinology **126:** 728–735
8 Schell D., Murphy T. C. and Samson W. K. (1995) Endothelin modulation of prolactin secretion. Frontiers in Endocrinology **1:** 145–152
9 Samson W. K., Martin L., Mogg R. and Fulton R. J. (1990) A non-oxytocinergic prolactin releasing factor and a non-dopaminergic prolactin inhibiting factor in bovine neurointermediate lobe extracts: in vitro and in vivo studies. Endocrinology **126:** 1610–1617
10 Samson W. K., Skala K. D., Alexander B. D., Huang F.-L. S. and Gomez-Sanchez C. (1992) The prolactin release inhibiting activity isolated from neurointermediate lobe extracts is an endothelin-like peptide. Regul. Peptides **39:** 103–112
11 Ivell R. and Russell J. A. (eds) (1995) Oxytocin: Cellular and Molecular Approaches in Medicine and Research, Plenum Press, New York
12 Lumpkin M. D., Samson W. K. and McCann S. M. (1983) Hypothalamic and pituitary sites of action of oxytocin to alter prolactin secretion in the rat. Endocrinology **112:** 1711–1717
13 Samson W. K., Lumpkin M. D. and McCann S. M. (1986) Evidence of a physiological role for oxytocin in the control of prolactin secretion. Endocrinology **119:** 554–560
14 Sarkar D. K. and Gibbs D. M. (1984) Cyclic variation of oxytocin in the blood of pituitary vessels of rats. Neuroendocrinology **39:** 481–483
15 Tashjian A. H., Barowsky N. J. and Jensen D. K. (1971) Thyrotropin releasing hormone: evidence for stimulation of prolactin production by pituitary cells in culture. Biochem. Biophys. Res. Commun. **43:** 516–523
16 Horn A. M., Fraser H. M. and Fink G. (1983) Effects of antiserum to thyrotropin releasing hormone on the concentrations of plasma prolactin, thyrotropin and luteinizing hormone in the pro-oestrous rat. J. Endocrinol. **10:** 205–209
17 Vijayan E., Samson W. K. and McCann S. M. (1979) Vasoactive intestinal peptide: evidence for a hypothalamic site of action to release growth hormone, luteinizing hormone, and prolactin in ovariectomized rats. Endocrinology 104: 153–159

18 Samson W. K., Said S. I., Snyder G. and McCann S. M. (1979) In vitro stimulation of prolactin release by vasoactive intestinal peptide. Peptides **1:** 325–332
19 Ruberg M., Rotsztejn W. H., Arancibia S., Besson J. and Enjalbert A. (1978) Stimulation of prolactin release by vasoactive intestinal peptide (VIP). Eur. J. Pharmacol. **51:** 319–320
20 Kato Y., Iwasaki Y., Abe H., Yanaihara N. and Imura H. (1978) Prolactin release by vasoactive intestinal polypetide in rats. Endocrinology **103:** 554–558
21 Said S. I., Porter J. C., Vijayan E., Samson W. K. and McCann M. (1978) VIP and hypothalamic-pituitary function. Scand. J. Gastroenterol. 13: 154
22 Samson W. K., Bianchi R., Mogg R. J., Rivier J., Vale W. and Melin P. (1989) Oxytocin mediates the hypothalamic action of vasoactive intestinal peptide to stimulate prolactin secretion. Endocrinology **124:** 812–819
23 Murai T., Reichlin S. R. and Ben-Jonathan N. (1989) The peak phase of the proestrous prolactin surge is blocked by either posterior pituitary lobectomy or antisera to vasoactive intestinal peptide. Endocrinology **124:** 1050–1055
24 Abe H., Engler D., Molitch M. E., Bollinger-Gruber J. and Reichlin S. (1985) Vasoactive intestinal peptide is a physiologic mediator of prolactin secretion in the rat. Endocrinology **116:** 1383–1389
25 Sakurai T., Amemiya A., Ishii M., Matsuzaki I., Chemelli R. M., Tanaka H. et al. (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell **92:** 573–585
26 Welch S. K., O'Hara B. F., Kilduff T. S. and Heller H. C. (1995) Sequence and tissue distribution of a candidate G-coupled receptor cloned from rat hypothalamus. Biochem. Biophys. Res. Commun. **209:** 606–613
27 Hinuma S., Habata Y., Fujii R., Kawamata Y., Hosoya M., Fukusumi S. et al. (1998) A prolactin-releasing peptide in brain. Nature **393:** 272–276
28 Langmead C. J., Szerkeres P. G., Chambers J. K., Ratcliffe S. J., Jones D. N. C., Hirst W. D. et al. (2000) Characterizations of the binding of [^{125}I]-human prolactin releasing peptide (PrRP) to GPR10, a novel G protein coupled receptor. Brit. J. Pharmacol. **131:** 683–688
29 Marchese A., Heiber M., Nguyen T., Heng H. H. Q., Saldivia V. R., Cheng R. et al. (1995) Cloning and chromosomal mapping of three novel genes, GPR9, GPR10 and GPR14 encoding receptors related to interleukin 8, neuropeptide Y and somatostatin receptors. Genomics **29:** 335–344
30 Samson W. K., Resch Z. T., Murphy T. C. and Chang J.-K. (1998) Gender-biased activity of the novel prolactin releasing peptides. Comparison with thyrotropin releasing hormone reveals only pharmacologic effects. Endocrine **9:** 289–291
31 Satoh F., Smith D. M., Gardiner J. V., Mahmoodi M., Murphy K. G., Ghatei M. A. et al. (2000) Characterization and distribution of prolactin releasing peptide (PrRP) binding sites in the rat – evidence for a novel binding site subtype in cardiac and skeletal muscle. Brit. J. Pharmacol. **129:** 1787–1793
32 Matsumoto H., Murakami Y., Hodikoshi Y., Noguchi J., Habata Y., Kitada C. et al. (1999) Distribution and characterization of immunoreactive prolactin-releasing peptide (PrRP) in rat tissue and plasma. Biochem. Biophys. Res. Commun. **257:** 264–268
33 Martinez de la Escalera G. and Weiner R. I. (1992) Dissociation of dopamine from its receptor as a signal in the pleiotropic hypothalamic regulation of prolactin secretion. Endocrine Rev. **13:** 241–255
34 Roland B. L., Sutton S., Wilson S. J., Luo L., Pyati J., Huvar R. et al. (1999) Anatomical distribution of prolactin releasing peptide and its receptor suggest additional functions in the central nervous system and periphery. Endocrinology **140:** 5736–5745
35 Chen C. T., Dun S. L., Dun N. J. and Chang J.-K. (1999) Prolactin-releasing peptide-immunoreactivity in A1 and A2 noradrendergic neurons of the rat medulla. Brain Res. **822:** 276–279
36 Iijima N., Kataoka Y., Kakihara K., Bamba H., Tamada Y., Hayashi S. et al. (1999) Cytochemical study of prolactin-releasing peptide (PrRP) in the rat brain. Neuroreport **10:** 1713–1716
37 Maruyama M., Matsumoto H., Fujiwara K., Kitada C., Hinuma S., Onda H. et al. (1999) Immunocytochemical localization of prolactin-releasing peptide in the rat brain. Endocrinology **140:** 2326–2333
38 Yamakawa K., Kudo K., Kanba S. and Arita J. (1999) Distribution of prolactin-releasing peptide-immunoreactive neurons in the rat hypothalamus. Neurosci. Lett. **267:** 113–116
39 Minami S., Nakata T., Tokita R., Onodera H. and Imaki J. (1999) Cellular localization of prolactin-releasing peptide messenger RNA in the rat brain. Neurosci. Lett. **266:** 73–75
40 Morales T., Hinuma S. and Sawchenko P. E. (2000) Prolactin-releasing peptide is expressed in afferents to the endocrine hypothalamus, but not in neurosecretory neurons. J. Neuroendocrinology **12:** 131–140
41 Lee Y. S., Yang P., Soares M. J. and Voogt J. L. (2000) Distribution of prolcatin-releasing peptide mRNA in the rat brain. Brain Res. Bull. **51:** 171–176
42 Lawrence C. B., Celsi F., Brennand J. and Luckman S. M. (2000) Alternative role for prolactin-releasing peptide in the regulation of food intake. Nat. Neurosci. **3:** 645–646
43 Matsumoto H., Noguchi J., Horikoshi H., Kawamata Y., Kitada C., Hinuma S. et al. (1999) Stimulation of prolactin release by prolactin-releasing peptide in rats. Biochem. Biophys. Res. Commun. **259:** 321–324
44 Tokita R., Nakata T., Katsumata H., Konishi S., Onodera H., Imaki J. et al. (1999) Prolactin secretion in response to prolactin-releasing peptide and the expression of the prolactin-releasing peptide gene in the medulla oblongata are estrogen dependent in rats. Neurosci. Lett. **276:** 103–106
45 Jarry H., Heuer H., Schomburg L. and Bauer K. (2000) Prolactin-releasing peptides do not stimulate prolactin release in vivo. Neuroendocrinology **71:** 262–267
46 Fuji R., Fukusumi S., Hosoya H., Kawamata Y., Habata Y., Hinuma S. et al. (1999) Tissue distribution of prolactin-releasing peptide (PrRP) and its receptor. Regul. Peptides **83:** 1–10
47 Swanson L. W. and Sawchenko P. E. (1980) Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. Neuroendocrinology **31:** 410–417
48 Chan R. and Sawchenko P. E. (1994) Spatially and temporally differentiated patterns of c-fos expression in brainstem catecholaminergic cell groups induced by cardiovascular challenges in the rat. J. Comp. Neurol. **348:** 433–460
49 Samson W. K., Resch Z. T. and Murphy T. C. (2000) A novel action of the newly described prolactin releasing peptides: cardiovascular regulation. Brain Res. **858:** 19–25
50 Matsumoto H., Maruyama M., Noguchi J., Horikoshi Y., Fujiwara K., Kitada C. et al. (2000) Stimulation of corticotropin-releasing hormone-mediated adrenocorticotropin secretion by central administration of prolactin-releasing peptide in rats. Neurosci. Lett. **285:** 234–238
51 Seal L. J., Small C. J., Kim M. S., Stanley S. A., Taheri S., Ghatei M. A. et al. (2000) Prolactin releasing peptide (PrRP) stimulates luteinizing hormone (LH) and follicle stimulating hormone (FSH) via a hypothalamic mechanism in male rats. Endocrinology **141:** 1909–1912
52 Watanobe H., Schioth H. B., Wikberg J. E. S. and Suda T. (2000) Evaluation of the role for prolactin-releasing peptide in prolactin secretion induced by ether stress and suckling in the rat: comparison with vasoactive intestinal peptide. Brain Res. **865:** 91–96
53 Maruyama M., Matsumoto H., Fujiwara K., Noguchi J., Kitada C. and Hinuma S. et al. (1999) Central administration of pro-

lactin-releasing peptide stimulates oxytocin release in rats. Neurosci. Lett. **276:** 193–196
54 Hinuma S., Onda H. and Fujino M. (1999) The quest for novel bioactive peptides utilizing orphan seven-transmembrane-domain receptors. J. Mol. Med. 77: 495–504
55 Yang H. Y. T., Fratta W., Majane E. A. and Costa E. (1985) Isolation, sequencing, synthesis and pharmacological characterization of two brain neuropeptides that modulate the action of morphine. Proc. Natl. Acad. Sci. USA **82:** 7757–7761
56 Aarnisalo A. A., Tuominen R. K., Nieminen M., Vainio P. and Panula P. (1997) Evidence for prolactin releasing activity of neuropeptide FF in rats. Neuroendocrinol. Lett. **18:** 191–196
57 Hinuma S., Shintani Y., Fukusumi S., Iijima N., Matsumoto Y., Hosoya M. et al. (2000) New neuropeptides containing carboxy-terminal RFamide and their receptor in mammals. Nat. Cell. Biol. **2:** 703–708

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