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# Novel Hormone "Receptors"

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**Abstract** Our concepts of hormone receptors have, until recently, been narrowly defined. In the last few years, an increasing number of reports identify novel proteins, such as enzymes, acting as receptors. In this review we cover the novel receptors for the hormones atrial naturetic hormone, enterostatin, hepcidin, thyroid hormones, estradiol, progesterone, and the vitamin D metabolites $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$. J. Cell. Biochem. 103: 401–407, 2008.


**Key words:** hormone receptors; 1,25-dihydroxyvitamin D; nongenomic action

In endocrinology, hormone receptors have been classically divided into two major groups: those for peptides and those for steroids. Within the peptide hormone group, serpentine (seven membrane domain) receptors, and receptors that activate soluble tyrosine kinases are common. For steroid hormones, receptors that function as transcription factors have been intensely studied. However, there are increasing reports of hormones binding to, and altering the activity of, unusual proteins. This review summarizes some of these.

## PEPTIDE HORMONES

Insulin and growth hormone, among others, bind to receptors that have intrinsic tyrosine kinase activity that initiate many signal transduction events. This, however, is just one example of a hormone binding to an enzyme.

## ATRIAL NATRIURETIC HORMONE

Blood volume and pressure are regulated by an endocrine system that affects both water and sodium resorption from the kidney. Conditions of hyponatremia and hypovolemia cause the release of renin which leads to the down stream production of angiotensin II, a potent vasoconstrictor, and activator of aldosterone production, which in turn enhances sodium resorption. Under conditions of hypervolemia and hypernatremia, the heart releases atrial natriuretic hormone (ANH) which inhibits both renin release and aldosterone production, causes blood vessel relaxation, and inhibits vasopressin release. ANH was subsequently reported to act through a membrane receptor of 180 kDa [Sharma et al., 1989; Marala and Sharma, 1991]. Subsequent purification to homogeneity revealed that the receptor was also a guanylate cyclase, distinct from soluble guanylate cyclases.

## ENTEROSTATIN

More recently, the satiety hormone enterostatin, has been found to be a pentapeptide cleaved from procolipase. Chronic treatment of rats with enterostatin decreases body weight and body fat. Park et al. [2004] have reported that the enterostatin receptor is the F1-ATPase beta subunit. Whereas no binding could be detected in the assembled F1-ATPase complex, the beta subunit, present on plasma and mitochondrial membranes of rat liver and amygdala, was found to have a $Kd = 150$ nM. This novel receptor suggested that enterostatin would have effects on energy metabolism. Indeed, systemic enterostatin reduced the respiratory quotient and increased metabolic rate

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in male rats. Intracerebroventricular enterostatin increased metabolic rate, while no effect was found when the peptide was injected into the amygdala [Lin et al., 2005].

## HEPCIDIN

Perhaps the most significant recent breakthrough in iron biology was the discovery of the peptide hormone hepcidin and its receptor ferroportin. There are no known regulated iron excretion pathways and because excess iron is toxic; regulation of dietary iron absorption from the intestine as well as iron flux from macrophages (which catabolize senescent erythrocytes and recycle iron) is paramount. It has been long known that dietary iron absorption is curtailed when iron stores are high [Stewart et al., 1950] and that plasma iron decreases during inflammation and/or infection [Cartwright et al., 1946; Greenberg et al., 1947]. However, the molecular mechanisms of how these processes are controlled were not fully understood until recently. Deemed as the "master regulator" of iron homeostasis, the peptide hormone hepcidin was discovered serendipitously when mice with a disrupted upstream regulatory factor 2 (USF2) gene developed massive iron overload [Nicolas et al., 2001]. In the initial USF2 knockout experiment, the hepcidin gene was unintentionally dirupted and implicated as the causative factor of the observed iron overload. The hepcidin gene encodes an 84 amino acid propeptide that is cleaved to form a bioactive 25 amino acid peptide found in plasma and urine [Krause et al., 2000; Park et al., 2001]. The active 25 amino acid peptide hormone forms a hairpin stabilized by four disulfide bonds [Hunter et al., 2002]. Truncation of the last five amino acids causes loss of function in vivo [Nemeth et al., 2006]. Hepcidin is synthesized in the liver and gene expression is increased by iron overload [Pigeon et al., 2001; Detivaud et al., 2005] and inflammation [Nemeth et al., 2003] and decreased by hypoxia and anemia [Nicolas et al., 2002]. Hepcidin expression is induced in inflammation by interleukin 6 [Nemeth et al., 2004a] and interleukin 1 [Lee et al., 2005] and induction is mediated by STAT3 transcription factor binding to the hepcidin promoter [Wrighting and Andrews, 2006; Verga Falzacappa et al., 2007]. Hepcidin expression results in dramatic decreases of plasma iron concentrations. Serum iron levels of mice injected with synthetic hepcidin dropped more than 400% within 1 h after injection and remained depressed for 48 h [Rivera et al., 2005]. Hepcidin gene deletion results in severe iron overload [Nicolas et al., 2001; Lesbordes-Brion et al., 2006] and mutations of the human hepcidin gene have been implicated in the etiology of some forms of the iron overload disease hemochromatosis [Delatycki et al., 2004; Majore et al., 2004; Roetto et al., 2004].

Hepcidin modulates iron metabolism through interactions with its receptor, ferroportin the only known cellular iron efflux protein. Ferroportin was discovered independently by three different groups using three different approaches [Abboud and Haile, 2000; Donovan et al., 2000; McKie et al., 2000]. Ferroportin is a basolateral, transmembrane protein [Liu et al., 2005] that is strongly expressed in placenta, intestine, reticuloendothelial macrophages and hepatocytes [Abboud and Haile, 2000; McKie et al., 2000; Donovan et al., 2005]. Ferroportin mobilizes iron from cells to the plasma iron carrier transferrin in conjuction with the ferroxidase protein hephaestin [Vulpe et al., 1999]. Overexpression of ferroportin has been demonstrated to increase iron efflux out of macrophages by 70% [Knutson et al., 2005]; conversely, ferroportin knockdown in macrophages increased cellular iron three- to eightfold [Galli et al., 2004]. Disruption of the murine ferroportin gene is embryonic lethal due to lack of iron transfer from the extraembryonic visceral endoderm to the placenta [Donovan et al., 2005]. Intestine specific knockout animals were viable but developed severe anemia.

Hepcidin induces irreversible internalization of ferroportin through lysomal degradation [Nemeth et al., 2004b]. Green fluorescent protein (GFP) tagged ferroportin expressed in HEK293 cells translocated from the cell surface to the lysosomes within 1 h of 0.1 μM hepcidin exposure. Similarly, macrophages exposed to hepcidin in culture had dramatic losses of ferroportin protein concentration after 3 h of exposure [Knutson et al., 2005]. The net effect of hepcidin expression and subsequent internalization of ferroportin results in a depletion of plasma iron and accumulation of iron in macrophages and duodenal eneterocytes. Accumulation of iron in these cells creates a feedback circuit; macrophages release less recycled erythrocyte iron and duodenal cells take up less

dietary iron which depletes plasma iron as this store is used to synthesize new hemoglobin. It appears that the hepcidin/ferroportin system is the key mechanism to limit iron availability to pathogens as a host defense strategy or increase plasma iron in response to anemia/hypoxia as well as limiting iron efflux from duodenal enterocytes in response to dietary iron overload.

## THYROID HORMONES

Thyroid hormones are integral to maintenance of the basal metabolic rate, and crucial in embryonic development. Not surprisingly, the nongenomic actions of thyroid hormones are numerous [Davis and Davis, 1996]. At least three binding proteins have been identified, one of which is pyruvate kinase $M_2$. Binding to the monomer prevents formation of the tetramer, and hence decreases activity of the enzyme. In addition, thyroid hormone (3,3′,5-triiodo-l-thyronine, or $T_3$) has been reported to bind to protein disulfide isomerase [PDI; Primm and Gilbert, 2001], an enzyme first identified as a chaperone in the endoplasmic reticulum. An isoform of this enzyme also serves as a receptor for other steroids (see below). Finally, the integrin $\alpha v\beta 3$ has been identified as a cell surface receptor for thyroid hormone and mediates activation of the mitogen-activated protein kinase (MAPK) signal transduction cascade [Cody et al., 2007]. A notable observation in this regard is that a deaminated thyroid hormone analog, tetraiodothyroacetic acid, inhibits binding of $T_4$ and $T_3$ to the integrin, and does not activate MAPK [Cody et al., 2007].

## STEROID HORMONES

While a number of the classical family of transcription factors have been found in association with the plasma membrane, numerous other reports indicate unrelated receptors are also on the cell surface [e.g., Christ et al., 1994; Eisen et al., 1994; Benten et al., 1999; Toran-Allerand et al., 2002; Boyan et al., 2003].

## SEX STEROIDS

Many rapid responses to estradiol have been attributed to the traditional estrogen receptor at the cell surface. In contrast, Valverde et al. [1999] found that estradiol bound directly to the regulatory ($\beta$) subunit of the Maxi-K channel. Modulation of this channel by estrogens is believed to contribute a protective effect to the cardiovascular system in pre-menopausal women.

When oocytes expressed only the pore forming $\alpha$ subunit of the Maxi-K channel, no effect of estradiol was seen in patch clamped cells, whereas oocytes expressing both subunits responded to estradiol with increased current recordings. The authors further found that such effects occurred in response to estradiol-BSA conjugates, but not to microinjected hormone.

A G-protein coupled receptor, GPR30, has also been found to act as a receptor for estradiol [Revankar et al., 2005]. Expression of a GFP construct (GPR30-GFP) in monkey kidney fibroblast cells (COS7), along with the $\beta_2$-adrenergic receptor demonstrated that while the latter localized to the plasma membrane, GPR30-GFP localized to the endoplasmic reticulum, including the Golgi. These results were paralleled by immunohistochemical studies. Moreover, this receptor protein was found to mediate estradiol dependent calcium mobilization, indicating functionality of protein–ligand interactions.

These two observations for estradiol, binding to channels and G-protein coupled receptors, are paralleled by other work done with progesterone. Progesterone was reported to bind to an extracellular part of the nicotinic acetylcholine receptor to cause inhibition [Valera et al., 1992] of neurotransmitter-evoked currents. Dose-response curves for progesterone-BSA conjugates paralleled those for free steroid. Inhibition by progesterone was also found to not require the presence of agonist, was voltage-independent, and did not alter receptor desensitization. The authors concluded that progesterone was not a competitive inhibitor, but may interact with the acetylcholine-binding site.

Progesterone functions in establishing and maintaining pregnancy in mammals. One of its maintenance functions is to decrease uterine sensitivity to oxytocin, a peptide hormone that promotes labor. Oxytocin acts through a G-protein coupled receptor to induce inositol phosphate production and calcium mobilization. Addition of progesterone to membranes derived from a parturient rat uterus inhibited binding of an oxytocin receptor specific ligand [Grazzini et al., 1999]. Progesterone likewise induced a dose-dependent reduction of specific

oxytocin binding to recombinant rat receptor expressed in CHO cells. Finally, progesterone induced a dose-dependent inhibition of calcium mobilization.

## VITAMIN D METABOLITES

Many studies have been conducted on the rapid, nongenomic responses to vitamin D metabolites, particularly 1,25-dihydroxyvitamin $D_3$ [1,25(OH))$_2D_3$] and 24,25-dihydroxyvitamin $D_3$ [24,25(OH)$_2D_3$]. The metabolite 1,25(OH))$_2D_3$ is made under conditions of calcium, phosphate, and vitamin D deficiency and acts to raise serum calcium and phosphate, in part through enhanced intestinal absorption of dietary minerals, and renal reabsorption of circulating minerals. Serum calcium levels are particularly tightly controlled, so it is logical that rapid responses occur. Using the chick model system resulted in the serendipitous discovery that 1,25(OH)$_2D_3$ uses PDIA3/ERp57 as the membrane associated receptor to initiate rapid calcium and phosphate uptake. The protein, which we refer to as the 1,25$D_3$-MARRS (membrane associated, rapid response steroid-binding) receptor was isolated on the basis of binding of isotopically labeled ligand [Nemere et al., 1994]. Using both antibodies (Ab 099 to 1,25$D_3$-MARRS and 9A7 to the classical vitamin D receptor, VDR) and RNAi we demonstrated that the 1,25$D_3$-MARRS receptor, and not the VDR is responsible for the nongenomic actions of 1,25(OH)$_2D_3$ in chick intestine [Nemere et al., 2004] and kidney [Khanal et al., 2007]. That this is truly a cell surface protein was demonstrated by fluorescence activated cell sorting [Teillaud et al., 2005]. Intriguingly, in rat intestine, rapid responses are mediated by both the 1,25$D_3$-MARRS receptor and cell surface VDR [Nemere, 2005]. This would suggest that the 1,25$D_3$-MARRS receptor is the evolutionarily more ancient system, and that mammals subsequently evolved use of cell surface VDR. However, rat matrix vesicles—likely responsible for bone mineralization—contain only the 1,25$D_3$-MARRS receptor [Boyan et al., 2007].

The metabolite 24,25(OH)$_2D_3$ is made when an animal is vitamin D replete, and provides an endocrine feedback loop to inhibit the rapid actions of 1,25(OH)$_2D_3$. Binding studies with [$^3$H]24,25(OH)$_2D_3$ in both isolated intestinal epithelial cells and subcellular fractions indicated a localization of the active moiety on the basal lateral membrane to a small extent, with a much larger pool in the vesicular fraction [Nemere et al., 2002]. Purification of the protein and sequencing led to the identification of catalase as the binding protein [Larsson et al., 2006]. Binding of ligand to catalase resulted in a decrease in enzyme activity within 1 min [Nemere et al., 2006] that was evident even 5 h later [Khanal et al., 2007]. The increase in $H_2O_2$ levels (approximately 30%) rapidly reduced [$^3$H]1,25(OH)$_2D_3$ binding to the 1,25$D_3$-MARRS receptor if the assay was conducted in the absence of dithiothreitol, suggesting the involvement of the thioredoxin domains on the protein [Nemere et al., 2006]. Binding to the VDR was not affected in the same samples. 1,25(OH)$_2D_3$-stimulated protein kinase C activity—which mediates the rapid phosphate uptake response—was likewise directly affected by increased $H_2O_2$ levels [Peery and Nemere, 2007]. Neutralization of the inhibitory effect of 24,25(OH)$_2D_3$ on 1,25(OH)$_2D_3$ enhanced phosphate uptake was accomplished by preincubating isolated enterocytes with either a commercially available anti-catalase antibody, or one generated in house [Larsson et al., 2006; Peery and Nemere, 2007].

As a final test of the functionality of this system, we raised chicks on a standard diet,

**TABLE I. Hormones and Their Novel Receptors**

| Hormone | Receptor | References |
|---|---|---|
| Atrial naturetic hormone | Guanylate cyclase | Sharma et al. [1989] |
| Enterostatin | F1 ATPase β subunit | Park et al. [2004] |
| Hepcidin | Ferroportin | Nemeth et al. [2004b] |
| Thyroid hormones | Pyruvate kinase $M_2$ | Davis and Davis [1996] |
| | Protein disulfide isomerase | Primm and Gilbert [2001] |
| | Integrin αvβ3 | Cody et al. [2007] |
| Estradiol | Maxi-K channel | Valverde et al. [1999] |
| | GPR30 | Revankar et al. [2005] |
| Progesterone | Oxytocin receptor | Valera et al. [1992] |
| 1,25(OH)$_2D_3$ | PDIA3/ERp57 "1,25$D_3$-MARRS" | Nemere et al. [2004] |
| 24,25(OH)$_2D_3$ | Catalase | Larsson et al. [2006] |

with somewhat reduced levels of vitamin D, or the same diet supplemented with either vitamin C or twice the level of vitamin E. Chicks on the diets supplemented with antioxidants had nearly twice the level of phosphate absorption in vivo as chicks on the standard diet [Nemere et al., 2006].

## CONCLUSIONS

We have summarized the hormones and novel receptors in Table I. The results presented above indicate that we will need to revise our view of how hormones act on cells, and perhaps even the definition of "receptor." While currently the concept of more than one "receptor" per hormone makes understanding of endocrine regulation greatly more complex, ultimately it will guide us in identifying the myriad ways in which pleiotropic endocrine effects occur. In the case of hormones that bind enzymes, we speculate that the cell surface appearance of these proteins, containing appropriate organelle targeting information, allows the corresponding hormone ligand to be directed to the subcellular site where it exerts its regulatory influence. In this context it is quite understandable that a hormone, such as enterostatin, that regulates metabolic energy would be targeted to the mitochondria. Likewise, the inhibitory hormone $24,25(OH)_2D_3$ is targeted to peroxisomes to promote $H_2O_2$ production that affects sensitive thiol groups. A further corollary is that identification of the enzyme "receptor" will allow insights into the hormone's mechanism of action.

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