Published in final edited form as:

J Neuroendocrinol. 2012 April ; 24(4): 554–565. doi:10.1111/j.1365-2826.2011.02268.x.

Diverse Roles of G-Protein Coupled Receptors in Regulation of Neurohypophyseal Hormone Secretion

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Abstract

The magnocellular neurons in the supraoptic nucleus project to the neural lobe and release vasopressin and oxytocin into the peripheral circulation where they act on the kidney to promote fluid retention or stimulate smooth muscles in the vasculature, uterus and mammary glands to support blood pressure, promote parturition, or induce milk let-down respectively. Hormone release is regulated by complex afferent pathways carrying information about plasma osmolality, blood pressure and volume, cervical stretch, and suckling. These afferent pathways utilize a broad array of neurotransmitters and peptides that activate both ligand-gated ion channels and G-protein coupled receptors (GPCRs). The ligand-gated ion channels induce rapid changes in membrane potential resulting in generation of action potentials, initiation of exocytosis, and release of hormone into the periphery. In contrast, the GPCRs activate a host of diverse signaling cascades that modulate action potential firing and regulate other cellular functions required to support hormone release (e.g. hormone synthesis, processing, packaging and trafficking). The diversity of these actions is critical for integration of the distinct regulatory signals into a response appropriate for maintaining homeostasis. This review will describe several diverse roles of GPCRs in magnocellular neurons focusing primarily on adrenergic, purinergic, and peptidergic (neurokinin and angiotensin) receptors.

Keywords

vasopressin; oxytocin; supraoptic nucleus; adrenergic; purinergic; neurokinin; angiotensin

INTRODUCTION

Vasopressin (VP) and oxytocin (OT) are produced by magnocellular neurons (MCNs) in the supraoptic (SON) and paraventricular nuclei (PVN), transported down the axons to the neural lobe and released into the blood stream in response to action potentials that depolarize the nerve terminals. VP acts on the kidney to increase water reabsorption and causes vasoconstriction to support blood pressure. The role of VP in maintaining fluid and electrolyte balance and supporting cardiovascular function is complex, because to accomplish these tasks, the MCNs must integrate information about extracellular fluid osmolality, blood pressure and volume, and body temperature. In addition, the oxytocinergic MCNs play a vital role in parturition and lactation. Osmolality is monitored directly by the MCNs, but information about osmolality is also transmitted from osmoreceptive cells located in the organum vasculosum of the lamina terminalis (OVLT). Furthermore, osmoreceptive cells located in the periphery provide early warnings of fluid or electrolyte

consumption. Blood pressure and volume is monitored in the carotid sinus and cardiac atrium and transmitted to the MCNs via multiple, multisynaptic pathways with the final pathways to SON and PVN employing GABA, ATP, and norepinephrine as the neurotransmitters (1-10). Similarly, suckling and cervical stretch initiate OT release via activation of poorly delineated multisynaptic circuits involving the afferents in the brainstem and spinal cord (11).

While all of these afferents employ 'fast acting' neurotransmitters such as glutamate, GABA, ATP, or acetylcholine that can rapidly alter neuronal firing rates by activating ionotropic receptors, the same neurotransmitters as well as co-released catecholamines and neuropeptides activate G-protein coupled receptors (GPCR) to regulate a variety of cellular responses needed for hormone release. Although our knowledge about the mechanisms by which the fast acting neurotransmitters generate action potentials is extensive (12, 13), it is probably the subtle modulation of MCN activities by GPCRs that underlies the ability of MCNs to integrate the diverse information provided by multiple afferent pathways into responses appropriate for maintaining homeostasis.

G-protein coupled receptors bind tri-heteromeric G-protein complexes that include α , β , and γ G proteins. Upon ligand binding, the $G\alpha$ protein becomes uncoupled from the $G\beta\gamma$ proteins allowing it to initiate cellular signaling cascades. As summarized in Fig. 1, GPCRs are divided into 4 families based on the $G\alpha$ subtype: $G\alpha_s$, $G\alpha_i$, $G\alpha_{q/11}$, or $G\alpha_{12/13}$. These subtypes activate distinct cell signaling cascades. Specifically, $G\alpha_s$ activates adenylyl cyclase resulting in an increase in cAMP and activation of protein kinase A (PKA); $G\alpha_i$ inhibits adenylyl cyclase reducing cAMP production; $G\alpha_{q/11}$ activates phospholipase C β (PLC) resulting in generation of the second messengers, diacylglycerol (DAG) which activates protein kinase C (PKC) and inositol triphosphate (IP3) which initiates release of Ca^{++} from intracellular stores; and $G\alpha_{12/13}$ regulates rho. The β/γ subunits associated with $G\alpha_i$ and $G\alpha_{q/11}$ also regulate cell processes including K^+ conductances when uncoupled from $G\alpha$ (14).

Over 100 non-chemosensory GPCRs have been detected in SON and/or PVN (15), and at least 40% of these signal through $G_{q/11}$ [(15); and see: <http://www.iupharb.org/>]. Thus, it may seem that there would be considerable redundancy in their effects. However, even GPCRs linked to the same $G\alpha$ subunit can induce dramatically different hormone release responses (Fig. 2), and have a wide variety of effects ranging from membrane depolarization to altered expression of ligand-gated ionotropic receptors. Factors that contribute to this diversity include coupling to more than one $G\alpha$ subtype, signaling initiated by the $G\beta/\gamma$ subunit, signaling via tyrosine phosphorylation, modulation of multiple types of ion channels (e.g. ligand-gated, K^+ , TRP, or Ca^{++}), and ligand-induced trafficking of the GPCR to the nucleus. This review will focus on GPCRs that have been investigated in this laboratory. Specifically, adrenergic, purinergic, neurokinin, angiotensin, and metabotropic glutamate and GABA receptors will be discussed as examples of the diverse mechanisms by which GPCRs give rise to different hormone release patterns. As a result, this is not an exhaustive or comprehensive review of known mechanisms of GPCR signaling in MCNs.

Adrenergic Receptors

Increase MCN firing—As one of the classic neurotransmitters, the role of norepinephrine in regulation of VP and OT release was addressed in early studies (16), and the ability of norepinephrine to activate multiple GPCRs with opposite effects became evident: α_1 -adrenergic receptors (α_1 -Rs) are excitatory while β -adrenergic receptors inhibit VP release (7, 16-23). This led to the subsequent use of selective agonists (e.g. phenylephrine for α_1 -Rs) to study MCN adrenergic responses. In fact, in early studies phenylephrine (PE) was found to be one of the most reliable ligands for inducing action potentials in SON neurons in

explants of the hypothalamo-neurohypophyseal system (HNS) and was used to correlate hormone release with electrical activity (23). As shown in Fig 2A, 100 μ M PE causes a small, but significant increase in VP release (24).

Modify purinergic receptor expression—The physiological importance of norepinephrine as a neurotransmitter regulating VP release was solidified by the finding that destruction of the afferent noradrenergic-A1 pathway that connects the ventrolateral medulla to the SON and PVN prevented MCN responses to hemorrhage-induced decreases in blood volume and pressure. However, in subsequent studies, it was found that α 1-adrenergic antagonists did not block the responses to hemorrhage, but rather the responses were prevented by a non-selective purinergic receptor antagonist (7, 10, 25). This suggested that ATP, co-released with norepinephrine from the A1 terminals (26), was capable of exciting SON neurons, and left the question: What is the role of norepinephrine? To address this, hormone release responses induced by ATP, PE, and combined exposure to ATP plus PE were compared using perfused explants of the HNS. It was discovered that the responses to either ATP or PE alone were small and transient while exposure to ATP with PE resulted in a potentiated response that was sustained for hours (24). Subsequent work demonstrated that the sustained component of the response is dependent upon α 1-R-mediated recruitment of functionally distinct ionotropic purinergic receptors (27). The ionotropic purinergic receptor family (P2X) is well suited for converting transient responses to sustained hormone release, because it includes subtypes that rapidly desensitize (within milli seconds) as well as subtypes that are highly resistant to desensitization (28). The sustained component of the response to PE and ATP requires activation of the non-desensitizing P2X subtypes, P2X2/3 and/or P2X7-R (27). Although additional work is required to fully elucidate the mechanisms by which α 1-R activation induces expression of the non-desensitizing subtypes, the synergistic increase in hormone release requires new gene expression as well as PKC-mediated protein phosphorylation (24), and it is accompanied by a sustained elevation in intracellular calcium ($[Ca^{++}]_i$) (29). These observations suggest that PLC-mediated phosphorylation and/or Ca^{++} -dependent signaling induced reorganization of existing receptors into heteromeric complexes (e.g. formation of P2X2/3 heteromers from P2X2 and 3 homomers) and new receptor synthesis are involved in transitioning to sustained hormone release. In addition, these non-desensitizing P2X receptors have been shown to open membrane pores large enough to allow small neuroactive molecules such as ATP and amino acids to exit the cell, gain access to the extracellular space, and potentially act as excitatory or inhibitory autocrine or paracrine signals. Thus, the ability of the α 1-Rs to induce functional expression of these pore-forming receptors further expands the adrenergic influence on cell regulatory mechanisms.

Three α 1-R subtypes have been identified, α 1A, B, and D, and all three subtypes are expressed in SON or PVN (30-32). Although α 1B is the most prominent α 1 subtype expressed in SON neurons (32), pharmacological approaches suggested that multiple α 1-R subtypes could support the sustained stimulation of neurohypophyseal hormone release by ATP (33). Thus, diversity amongst the α 1-Rs in mediating this effect was not apparent.

In conclusion, α 1-R can both directly increase firing rate of MCNs resulting in increased hormone release and can enhance the action of a fast acting neurotransmitter, ATP, by altering the predominant type of ligand-gated ion channel, in this case ionotropic purinergic receptors, involved in generating hormone release. Thus the α 1-R is an example of a $G_{q/11}$ linked GPCR that both modifies the membrane potential resulting in rapid changes in hormone release and induces extended changes in responsiveness of the MCNs to another neurotransmitter.

Purinergic GPCRs

In addition to ionotropic receptors (the P2X-Rs discussed above), the purinergic receptor family also includes GPCRs, the P2Y_{1,2,4,6,11,12,13,14}-Rs. Of these, P2Y₁-R is prominently expressed in SON, and P2Y₂ and P2Y₄ are also expressed in a few SON neurons (34). P2Y₁ is a G_{q/11}PCR (35) and as anticipated from the known coupling of G_{q/11} to PLC, the activation of P2Y₁-Rs by a selective P2Y₁-R agonist, 2-methylthio-ADP (2-MeSADP), induced release of Ca⁺⁺ from intracellular stores resulting in transient increases in [Ca⁺⁺]_i (34) as well as transient increases in VP and OT release (36). Although the Ca⁺⁺ and hormone responses (Fig. 2B) are similar to those obtained by activation of the adrenergic α₁-G_{q/11}PCRs [Fig. 2B;(24, 27)], other downstream actions of these two types of G_{q/11}PCRs diverge as demonstrated by the fact that simultaneous activation of P2X and P2Y₁-Rs by ATP does not induce a sustained increase in hormone release comparable to that observed when ATP is presented with PE to activate α₁-Rs (24). Thus, activation of P2Y₁-Rs does not recruit non-desensitizing ligand-gated ion channels [e.g. P2X_{2/3} or 7 as occurs with α₁-R activation (29)]. Thus, this is a clear example of divergent actions of G_{q/11}PCRs.

Although P2Y₁-Rs do not alter expression of the P2X-R subtypes, they may have important actions on a different class of Ca⁺⁺-permeable, ion channels, the TRPV channels. This possibility is supported by the observation that about 20% of the increase in [Ca⁺⁺]_i induced by 2-MeSADP was due to influx of external Ca⁺⁺ (36). P2Y-Rs have been shown to activate TRPV1 receptors in Neuro 2a cells (37). A modified TRPV1 channel is expressed in SON and is proposed to be the molecular counterpart of the stretch-inactivated non-selective ion channels that mediate osmoreceptivity in SON neurons (38). Thus, the potential for ATP activation of P2Y-Rs to modulate activity of these channels may be physiologically important as a mechanism allowing MCNs to integrate osmotic and hemodynamic information. Several other ligands of G_{q/11}PCRs have been shown to modulate activity of the stretch-inactivated channels in SON. These include angiotensin II, neurotensin, and cholecystokinin (39, 40) all of which activate G_{q/11}PCRs. Since TRPV1 can be modulated by the lipid messenger, diacylglycerol (DAG), generated in response to uncoupling of G_{q/11} (41), it is possible that this is the mechanism which mediates angiotensin modulation of osmotically stimulated VP release (42, 43). Thus, these are examples of G_{q/11}PCRs modulating a second class of ion channels, the TRPV channels.

Neurokinin Receptors

Neurokinin receptors mediate the effects of the tachykinins, substance P (SP), Neurokinin A, and Neurokinin B. There are 3 subtypes of neurokinin receptors, NK₁, NK₂, and NK₃. SP has the highest affinity for NK₁, neurokinin A for NK₂, and neurokinin B for NK₃. However, the ligands are promiscuous and at higher concentrations, each tachykinin can activate the other NK-receptors. Expression of both NK₁ and NK₃ has been demonstrated in SON (15), and antidiuresis has been induced by injection of SP into SON or an NK₃ agonist, senktide, into PVN (44, 45). The neurokinin receptors are an appropriate focus for this review, because: 1) They have been shown to activate multiple G-protein signaling cascades; 2) they regulate K⁺ channels; and 3) the NK₃ receptors traffic to the nucleus.

Activation of multiple G-protein signaling cascades—The NK-Rs are GPCRs that have been shown to increase both phosphatidylinositol hydrolysis and cAMP formation suggesting that they are linked to both G_{q/11} and G_s (46). PLC activation occurs at a lower agonist concentration than activation of adenylyl cyclase with approximately an order of magnitude more ligand required to increase cAMP (46). Thus, while ligand activation of G_{q/11} is more efficient than G_s, tachykinins have the potential to activate multiple signaling cascades particularly when released into the confined space of a synaptic cleft that could result in high ligand concentrations.

While additional studies are required to confirm that SP activates both PLC and adenylyl cyclase in SON neurons, our studies on the effect of SP on neurohypophyseal hormone release from HNS explants are consistent with concentration-dependent activation of multiple signaling cascades (47). The VP response to SP is shown in Fig. 2C. At 1 μ M, SP caused a small, but significant increase in VP release from HNS explants, but 10 μ M SP induced a 400% increase in VP release. OT release was similarly effected (47). NK3-R antagonists, SB235375 or SB222200 (48) did not block the effect of SP on VP release (49). Thus, recruitment of NK3-R activation is unlikely to explain the response to the 10 μ M SP. Since cAMP is a potent stimulus for VP release from HNS explants (50), the dramatic increase in VP release observed with a 10-fold increase in SP, is consistent with recruitment of Gs coupled receptors and activation of adenylyl cyclase.

The ability of SP to initiate a large and sustained increase in VP secretion could have physiological importance, because SP is co-expressed in a subset of the A1 catecholamine neurons. As described above, this pathway is required for activation of SON neurons in response to decreases in blood pressure and blood volume, and these stimuli are known to induce the large and sustained elevations in plasma VP (pVP) that are required to prevent hemorrhagic shock (51). Large increases in pVP also occur in response to moment to moment postural changes such as moving from sitting to standing (52), and protect against orthostatic hypotension. Similar to PE, SP also augmented VP release to ATP (47), but further work is required to determine if this is $G_{q/11}$ - or G_s -mediated, and to determine if NK1 activation recruits the non-desensitizing, pore-forming P2X-Rs similar to PE. Regardless of the underlying mechanism, augmentation of the ATP response by both SP and PE is consistent with biological systems conserving redundant mechanisms to support critical physiological functions such as sustaining increased VP secretion during situations in which changes in water balance are required to correct blood loss.

Other GPCRs expressed in SON and/or PVN are also associated with activation of multiple $G\alpha$ proteins. Similar to NK1, the G-protein activated oestrogen receptor, GPER or GPR30, and the neuropeptide S receptor, NPS, are coupled to both $G_{q/11}$ and G_s . GPER is strongly expressed in OT MCN neurons in SON and PVN, but was not detected in VP neurons (53). Since oestrogen effects on OT and VP release from HNS explants can be attributed to oestrogen receptor β (54), a classical nuclear steroid receptor, studies using the specific agonist for the GPER, G-1, are required to determine the effect of GPER on OT release. NPS is expressed in SON and PVN (15). It has been reported to stimulate the hypothalamo-pituitary-adrenal axis and to inhibit food intake (55), but its role in regulating neurohypophyseal hormone secretion has not been evaluated.

The angiotensin II receptor, AT1, also couples to multiple $G\alpha$ proteins, but in contrast to NK1 and 3, it couples to $G_{q/11}$ and G_i (56). The majority of AII actions are attributable to PLC activation (56), however, since release of the β/γ proteins from G_i can activate PLC β 1, this could be due to $G_{q/11}$ and/or G_i activation (56). Consistent with PLC β activation of IP3, AII increases $[Ca^{++}]_i$ in SON neurons. The increase in $[Ca^{++}]_i$ is conserved in the absence of extracellular Ca^{++} indicating that AII stimulates release of Ca^{++} from intracellular stores (Song, unpublished observations). AII also stimulates VP release (57) and causes depolarization of SON neurons (58). Several other GPCRs expressed in SON and PVN also couple to both $G_{q/11}$ and G_i . These include the galanin receptor, Gal2, the melanin-concentrating hormone receptor, MCH1, and the orexin receptor, OX1 (15). Thus, each of these receptors has the potential to inhibit adenylyl cyclase in addition to activating PLC and increasing $[Ca^{++}]_i$.

SP inactivates K^+ channels [inwardly rectifying K^+ (Kir) and tandem pore (K_{2p})]
— K^+ channels play a primary role in determining the membrane potential, excitability, and

firing frequency of neurons. Over 100 genes encoding K^+ channel subunits exist in the human genome that give rise to 4 functional groups of K^+ channels: 1) Ca^{++} -activated (K_{Ca} or BK, IK and SK for big, intermediate, and small conducting K_{Ca}), 2) inwardly rectifying (K_{ir}), 3) tandem pore (K_{2p} ; subtypes also called TWIK, TREK, TASK, TALK, and THIK), and 4) voltage-gated (K_v). Members of each class of K^+ channels participate in determining the shape of the action potential, neuronal excitability, or firing frequency. In SON, action potentials are characterized sequentially by 1) a depolarizing spike, 2) a repolarization phase, 3) a hyperpolarization phase in which repolarization overshoots the resting membrane potential resulting in a hyperpolarized after potential (HAP); and 4) a depolarizing after potential (DAP) associated with increased excitability which promotes the next action potential (59). Also, following a train of action potentials, a long-lasting hyperpolarization of the membrane occurs called the after-hyperpolarizing potential (AHP) that limits burst duration and frequency (59). Specific K^+ channels have been associated with each of these characteristics: K_v channels give rise to the A- and K-currents (I_A and I_K) that contribute to the rate of repolarization of the action potential and to the HAP; SK channels are responsible for AHP; TASK-1, a K_{2p} channel, may underlie the DAP; and K_{ir} channels play a primary role in setting membrane potential and thereby influence neuron excitability (59). Inactivation (closing) of K^+ channels results in membrane depolarization and increased probability of action potential firing while activation (opening) results in hyperpolarization and blocking of action potentials. Many of these K^+ channels are subject to modulation by GPCRs.

SP has been shown to inactivate multiple K^+ channels in other neurons. In nucleus basalis, SP activation of PLC has been shown to depolarize neurons by inactivation of a K_{ir} (K_{ir3}). K_{ir3} is regulated by G-proteins and therefore referred to as GIRK (60). In neurons, GIRK consists of variable heterotetramers of the GIRK1,2, or 3 subtypes (61). GIRKs are inactivated by $G_{q/11}$ stimulation of PLC, but they are activated by G_i associated β/γ subunits (62, 63). Therefore, GIRK serves as an effector for both inhibitory and stimulatory responses to GPCR ligands, and mediates the actions of numerous neurotransmitters and neuropeptides in the brain. Similarly, activation of $\alpha 1$ -adrenergic receptors has been linked to GIRK inactivation in locus coeruleus (64), and activation of group 1 $G_{q/11}$ -coupled metabotropic glutamate receptors has been shown to increase excitability of MCNs by attenuation of a resting potassium conductance (65). Thus, it is possible that NK1, $\alpha 1$ -Rs, and mGluR act similarly in SON to modify GIRK. All three GIRK subtypes are prominently expressed in MCNs (66, 67). In a heterologous expression system, the action of SP has been shown to involve direct binding of $G_{q/11}$ to GIRK (68, 69), and thus, can likely occur independent of IP3 formation and release of Ca^{++} from intracellular stores. This would be consistent with our experience that SP did not reliably induce increases in $[Ca^{++}]_i$ in SON neurons (unpublished observation). This is distinct from our experience with ligands for other $G_{q/11}$ PCRs such as PE ($\alpha 1$ -receptor agonist) (33), 2-MeSADP (P2Y1 receptor agonist) (36), and AII activation of AT1 receptors [unpublished observation; (70)]. Thus, $G_{q/11}$ PCRs may induce slow depolarization, firing, and hormone release by direct inhibition of GIRK, and their action to increase $[Ca^{++}]_i$ may regulate other cell activities (e.g. gene expression, receptor trafficking, etc) that modulate responses to extracellular ligands.

SP also inactivates one of the tandem pore K^+ channels, TASK-1, in hypoglossal motoneurons (71). TASK-1 is one of 8 K_{2p} channels demonstrated to contribute to the basal membrane potential in SON (72). Thus, SP inactivation of TASK-1 in SON could also promote increased hormone release by membrane depolarization. Although multiple $G_{q/11}$ PCRs may have similar effects on GIRK, overall, each one may have a specialized role in regulating MCNs by virtue of modulating activity of overlapping, but distinct groups of ion channels.

NK3-R traffics to nucleus—In addition to activation of both PLC β and cAMP, ligand activation of the NK3 receptor results in the appearance of NK3-immunoreactivity (-ir) in the nucleus of magnocellular neurons in both SON and PVN (49, 73). This is observed in response to both hypotension and hyperosmotic stimulation (49, 73). It was also observed in response to a specific agonist for NK3 receptors, senktide, but was not induced by injection of SP in SON (49). NK3-ir appears in the nucleus within 5 minutes after local ligand activation of NK3 and remains in the nucleus for at least 1 hour (49). NK3 has a nuclear localization sequence in the C-terminal region (49), and it associates with a nuclear chaperone protein, importin- α -1 (74) which may facilitate its movement through a nuclear pore. The absence of similar nuclear localization sequence in the NK1 receptor (75, 76) may explain the specificity of nuclear trafficking to NK3. The full length NK3 molecule enters the nucleus as demonstrated by the recognition of NK3-ir protein in nuclear fractions from SON and PVN with antibodies directed at sequences in the N-terminus, 2nd extracellular loop, and C-terminus of the molecule (77). As shown in Fig 3, the NK3-ir in the nucleus has a clustered appearance which is distinct from the uniform staining observed for typical transcription factors such as Fos or oestrogen receptor α or β (78, 79). Using electron microscopy and co-immunoprecipitation, Flynn et al (80) show that NK3 is co-localized with histone H4 and acetylated H4 in PVN nuclei from osmotically challenged rats, and is complexed with acetylated Histone H3 and H4 in nuclear fractions from PVN. Acetylation of histones is associated with relaxation of the chromatin structure making it accessible to transcription factors and is generally associated with transcription activation (81, 82). Thus, ligand induced trafficking of NK3 receptors to the nucleus may facilitate regulation of genes important for increased hormone secretion during physiological challenges requiring extended secretion of neurohypophyseal hormones.

Localization of NK3 to the nucleus is not unique to the magnocellular neurons. Although ligand-induced translocation of NK3 to the nucleus has only been demonstrated in SON and PVN, similar nuclear staining patterns have been reported in globus pallidus, substantia nigra, and the ventral tegmental area (83-85), and western blot analysis of nuclear fractions revealed NK3-ir in multiple brain regions including caudate, lateral septum, bed nucleus of stria terminalis, amygdala, medial preoptic area, and ventral tegmentum (77). The broad distribution of NK3 in the brain and its localization to brain regions involved in functions as diverse as motivated behavior, mood, and blood pressure regulation suggests that NK3 translocation to the nucleus may have broad functional implications for CNS function.

Although direct signaling from the membrane to the nucleus is not classically associated with GPCR signaling, NK3 receptors are not the only GPCR that has been shown to localize to the nucleus. AT1 was the first GPCR shown to translocate to the nucleus in primary cultures of brain neurons (86), and recently evidence for nuclear localization of the oxytocin receptor (OTR) has been reported in cultures of several human cell lines (87). Interestingly, in these studies, the nuclear localization pattern of AT1 and OTR had the same clustered appearance that characterizes NK3-R in the nucleus (Fig. 3). AT1 was shown to stimulate expression of the norepinephrine transporter and tyrosine hydroxylase genes, and these effects were prevented by interfering with nuclear translocation of AT1 (86). Since both AT1 and OTR are expressed in SON and PVN, direct signaling from the membrane to the cell nucleus represents a potential mode of cell regulation by other GPCRs in VP and OT neurons.

AT1 Angiotensin Receptor: Epitomy of versatility!

As is evident from the above discussion, the AT1 receptor is the quintessential example for multi-faceted GPCR signaling. While most of the actions of AT1 can be attributed to its coupling to G_{q/11}, it also couples to pertussis-sensitive, G_i in several peripheral target tissues

(56). Evidence for modulation of TRPV channels by AII expands its influence to modulation of osmoreceptivity and thermosensitivity of MCNs. Furthermore, although not yet demonstrated in vivo, its ability to translocate to the nucleus suggests that it may play a direct role in regulation of gene expression. In addition to these actions discussed above, *AT1 promotes tyrosine phosphorylation and transactivation of growth factor receptors* (e.g. epidermal growth factor receptor) with resulting activation of mitogen-activated protein kinases (MAPK/ERK) (56, 88). These actions of AT1 are thought to mediate its effects on cell growth in the heart, kidney and vasculature, and have not been evaluated in SON or PVN. Therefore, it remains to be demonstrated that this plays a role in regulation of neurohypophyseal hormone secretion. Nevertheless, it represents a mechanism of action that should be considered, because other GPCRs have also been shown to initiate tyrosine phosphorylated-mediated actions.

Fast acting neurotransmitters also activate GPCRs

The importance of GPCRs as modulators of neuronal responses is further demonstrated by the fact that all of the fast acting neurotransmitters, meaning those that activate ionotropic receptors, also activate GPCRs. This includes glutamate, GABA, acetylcholine, and ATP. GPCRs activated by GABA and glutamate, are some of the most highly expressed GPCRs in SON and PVN based on array studies (15). In the case of glutamate, these are referred to as metabotropic glutamate receptors (mGlu-R) to distinguish them from the ionotropic glutamate receptors, and several mGlu-R subtypes, mGlu1,3,4,5, and 7, are expressed in SON and PVN (15). These are classified into groups I, II, or III based on sequence homology, pharmacology, and effectors (89). Group I mGluRs, mGlu-R1 and 5, couple to $G_{q/11}$ while Group II and III mGluRs couple to G_i (mGlu-R3, 4, and 7). As mentioned above, activation of group I mGluRs attenuates a resting potassium conductance (65). This is consistent with $G_{q/11}$ inhibiting GIRK. Activation of group I mGluRs also reduces the K^+ current, I_A , and SK (responsible for AHP) in subpopulations of SON neurons (59, 65). Activation of group I mGluRs increases VP and OT release (90). A group II/III agonist, L(+)-2-amino-4-phosphonobutyric acid (L-AP4) did not change basal VP or OT release of VP or OT nor inhibit osmotically stimulated hormone release (90).

GABA is a dominant inhibitory transmitter in SON and PVN (12). It activates both ionotropic ($GABA_A$) and metabotropic ($GABA_B$) receptors. Functional $GABA_B$ receptors are heterodimers of $GABA_{B1}$ and $GABA_{B2}$ subunits. Both subunits are prominently expressed in SON and PVN and are clustered at postsynaptic sites (91). $GABA_B$ is coupled to G_i (92). Its activation inhibits voltage-activated Ca^{++} currents and opens K_{ir} channels in MCNs (93-95). GABA exerts tonic inhibition of VP release from HNS explants, but the relative contribution of ionotropic and metabotropic receptors was not determined (96). Recently, a switch in the influence of $GABA_A$ activation from inhibition to excitation has been observed in SON during chronic osmotic stimulation of VP release (97). This is caused by an increase in E_{GABA} induced by increased expression of $Na(+)-K(+)-2Cl(-)$ cotransporter 1 (NKCC1) in MCNs (97). It will be interesting to determine if the effect of $GABA_B$ is also altered during chronic hypertonicity, because without modulation of the effect of GABA at these receptors, the reversal of $GABA_A$ might be neutralized.

Acetylcholine is the final example of a fast acting neurotransmitter for which both ionotropic and metabotropic receptors are expressed in MCNs. Prominent α -bungarotoxin binding exists in SON indicative of high expression of the ionotropic $\alpha 7$ -nicotinic receptor (98-101). Muscarinic receptors are the metabotropic cholinergic receptors and expression of one $G_{q/11}$ subtype, M3, was found in SON by microarray (15). Although cholinergic stimulation of VP release from HNS explants primarily reflected activation of nicotinic receptors (102, 103), there is evidence that muscarinic receptor activation promotes phasic firing in MCN (104). This reflects inhibition of the AHP by a K_{Ca} channel, and

downregulation of I_A (104, 105). Thus, acetylcholine has the ability to both initiate action potential firing by activation of nicotinic ionotropic receptor activation and modulate neuronal firing via cholinergic GPCRs.

Conclusion

As described above and summarized in Table 1, versatility in signaling by GPCRs can include differences in the $G\alpha$ subtype, coupling to multiple $G\alpha$ subtypes, signaling by the $G\beta/\gamma$ subunit, G-protein independent signaling, trafficking to the nucleus, and activation of a variety of ion channels including K^+ channels involved in determining membrane potential, neuronal excitability, firing rate, and discharge patterns, voltage-sensitive Ca^{++} channels, ligand-gated ion channels, and mechano/thermosensitive ion channels belonging to the TRPV family. This diversity of actions provides GPCRs with the capability to integrate physiological responses to the two homeostatically critical but distinct physiological regulators of VP and OT release: osmolality and volume of the extracellular fluid. Since these parameters are monitored by different receptors and the information is transmitted to the MCNs by distinct pathways, much of the integration of responses occurs within SON and PVN. GPCR ligands such as norepinephrine, SP and ATP released by brainstem pathways carrying information about blood volume and pressure have the potential to modulate excitability of the MCNs by altering K^+ and/or TRPV channel activity respectively, and thus alter the responsiveness of the MCNs to excitatory transmitters as well as locally monitored changes in osmolality and temperature. Activation of AT1 receptors similarly has been shown to alter the osmosensitive channel activity thought to be mediated by modified TRPV channels (38-40), and AII is recognized to potentiate osmotically-stimulated VP release (42). Integration of osmotic and volume stimuli is known to occur in vivo (106, 107). Hyponatremia occurs under conditions associated with altered or inappropriate integration of the osmotic and hemodynamic regulation of VP secretion (108, 109).

This review has not discussed several other aspects that can be important determinants of GPCR regulation of VP and OT release. One of these, membrane compartmentalization is particularly germane to the efficacy of GPCRs in regulating particular ion channels. The possibility that $G_{q/11}$ and G_i directly regulate GIRK requires proximity of the GPCR and GIRK in the membrane. The localization of GPCRs in membrane caveolae (110) supports the likelihood that versatility in their actions reflects their proximity to other signaling proteins such as PLC, adenylyl cyclase, kinases, GIRK, Ca^{++} -channels, TRPV, etc. Micro domain compartmentalization can result in specificity in protein phosphorylation as well as ion channel regulation. For example, subcellular localization of voltage-gated Ca^{++} -channels with K_{Ca} channels likely explains activation of BK in the absence of bulk changes in intracellular Ca^{++} (59). Micro domain compartmentalization may also prevent cross-talk between receptors utilizing the same $G\alpha$ signal cascade.

GPCRs have been shown to form heteromeric complexes that may activate different signaling cascades and regulate different cellular responses than homomeric GPCRs (111-113). As discussed above, heteromeric ionotropic P2X receptors are well described and differ in their desensitization rates from their homomeric constituents (28). For some GPCRs (e.g. $GABA_{B1}$ and $GABA_{B2}$) heteromerization is required for membrane expression and therefore for functional signaling, but other GPCRs form heteromers with receptors for a different ligand. For example, adenosine A1 receptors couple with P2Y1 or D1 dopamine receptors, AT1 with B2 bradykinin receptor, NK1 with μ -opioid receptor, $\alpha 1$ -adrenergic receptors with D1 dopamine receptors or H1 histamine receptors, and vasopressin V1A receptor with OTR (111). Since all of these GPCRs are expressed in MCNs as well as many others (15), the potential for diverse responses to specific GPCR ligands seems almost limitless!

We also have not discussed the impact of experimental techniques on available information. For example, we have used whole cell Ca^{++} measurements to understand mechanisms of GPCR signaling in MCNs, but this has limitations as the technique may not detect physiologically relevant Ca^{++} changes in cellular microdomains. Thus, physiologically important Ca^{++} responses may not be detected. This emphasizes the limitations of drawing conclusions from negative results. We also have employed the HNS explant extensively to study the effect of neurotransmitters and neuropeptides on hormone release. While this preparation has been extremely useful, it is not possible to dissect postsynaptic, presynaptic, glial, and terminal actions of the ligands. Thus, some of the diversity that we detect in hormone release in response to $\text{G}_{q/11}$ agonists, for example, may reflect differences in their presynaptic and/or glial actions. For example, norepinephrine has presynaptic and glial actions as well as postsynaptic actions (30, 114) that may contribute to the actions of PE on hormone release. While it is possible to exclude some of these effects using tetrodotoxin to block Na^+ channels in Ca^{++} -imaging and electrophysiology studies, this approach is not possible in the hormone release studies that rely on generation of action potentials for hormone secretion. Another issue is the use of heterologous expression systems to study signaling cascades. While these are useful for identifying potential actions of GPCRs, the results need to be confirmed in MCNs, because expression of proteins involved in the signal cascades and/or their cellular compartmentalization may be cell type specific.

The focus of this review on adrenergic, purinergic, neurokinin, angiotensin, and metabotropic glutamate and GABA GPCRs only touches on a few of the 100 GPCRs expressed in SON and PVN and therefore, just represents the tip of the iceberg relative to mechanisms available for diverse and subtle modulation and integration of MCN function by GPCRs.

Acknowledgments

This research was supported by NIH grants RO1-NS27975, RO1-NS44835, R21-NS59569, and American Heart Association Grant-in-Aid 0855728G to CDS and American Heart Association Scientist Development Grant 0735329N to ZS. Many thanks to the colleagues who contributed to this work including Wanida Stevens, Dayane Gomes, Heather Howe, John Kapoor, Delmore Morsette, Francis W. Flynn, Dane D. Jensen, and William E. Armstrong.

List of Abbreviations

$\alpha 1$-R	alpha 1 adrenergic receptor
AII	angiotensin II
AHP	after hyperpolarizing potential
AT1	angiotensin 1 receptor
ATP	adenosine triphosphate
BK	big conducting K_{Ca}
cAMP	cyclic adenosine monophosphate
DAP	depolarizing after potential
GABA	gamma amino-butyric acid
GIRK	G-protein sensitive, inwardly rectifying K^+ channel
GPCR	G-protein coupled receptor
HAP	hyperpolarizing after potential

HNS	hypothalamo-neurohypophyseal system
IK	intermediate conducting K_{Ca}
IP3	inositol triphosphate
K_{2P}	tandem pore K^+ channel
K_{Ca}	calcium activated K^+ channel
Kir	inwardly rectifying K^+ channel
K_v	voltage sensitive K^+ channel
MCNs	magnocellular neurons
NK1 and 3	neurokinin 1 and 3 receptors
NPS	neuropeptide S receptor
OT	oxytocin
OTR	oxytocin receptor
OVL	organum vasculosum of the lamina terminalis
PE	phenylephrine
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PVN	paraventricular nucleus
SK	small conducting K_{Ca}
SON	supraoptic nucleus
SP	substance P
VP	vasopressin

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Figure 1.

Diagram of the classic G-protein signaling cascades. Shown is the 7 transmembrane G-protein coupled receptor (GPCR) coupled to the trimeric $G\alpha\beta\gamma$ complex, and the divergent effects of activation of $G\alpha_s$, $G\alpha_i$, $G\alpha_{q/11}$, $G\alpha_{12/13}$ and $G\beta\gamma$. $G\alpha_s$ activates adenylylase resulting in production of cAMP and activation of protein kinase A (PKA) while $G\alpha_i$ has the opposite effect due to inhibition of adenylylase. The β/γ subunit associated with $G\alpha_i$ opens a G-protein regulated K^+ channel (GIRK) resulting in membrane hyperpolarization. $G\alpha_{q/11}$ activates phospholipase C β (PLC β) which in turn activates inositol triphosphate (IP3) which binds to receptors on the endoplasmic reticulum resulting in release of stored Ca^{++} . PLC β also initiates formation of diacylglycerol (DAG) which activates protein kinase C (PKC). The β/γ subunit associated with $G\alpha_{q/11}$ also regulates cell activity. This may require intermediate signals. $G\alpha_{12/13}$ regulates Rho.

Figure 2.

Comparison of the VP response to ligands for three different $G_{q/11}$ PCR. Explants of the hypothalamo-neurohypophyseal system (HNS) were maintained in a perfusion system, exposed to different ligands, and VP concentration in the effluent was determined by radioimmunoassay. A. VP release in response to phenylephrine (100 μ M), an α 1-adrenergic receptor agonist. B. VP release in response to addition of the P2Y1 agonist, 2-Me-SADP. C. VP release in response to SP (1 and 10 μ M). Note the compression of the Y-axis in C. Ligands were added at the times indicated by the arrows and were present throughout the remainder of the perfusion. Note that while the response to 1 μ M SP was of similar magnitude to that induced by phenylephrine and 2Me-SADP, the response to 10 μ M SP was much larger. Also, the response to 2Me-SADP was delayed compared to either phenylephrine or SP, and responses to SP and 2Me-SADP were more sustained than the phenylphrine response. Explants were allowed to equilibrate for 4 hours prior to addition of the ligand, and VP release is expressed as % of basal release measured at the end of the 4 hr equilibration period. * $p < 0.05$; ** $p < 0.001$. Adapted with permission from (24, 36, 47).

Figure 3.

Neurokinin 3 (NK3-R)-immunoreactivity (ir, green) in magnocellular neurons of supraoptic neurons. In this single confocal plane from a rat made hypotensive with hydralazine, NK3-R-ir is present in the cytoplasm indicating internalization of receptor from the membrane to the cytoplasm in response to hypotension-induced release of a NK3-R ligand. Prominent NK3-R-ir (arrows) is also present in the nucleus (blue nuclear stain is DAPI). The presence of the nucleolus and confocal optical sectioning of the specimen supports the interpretation that the NK3-ir is inside the nucleus. Nuclei without NK3-R-ir are also present (asterisks). For more information and quantitation of the effect of hypotension and senktide on nuclear localization of NK3-R see (49, 77).

TABLE 1

Summary of the diverse actions of GPCRs discussed herein.

G _{q/11} PCR	Effect in MNCs*	Diverse Actions				Tyrosine Phosphorylation
		Ion Channel	Multiple G _α coupling	β/γ effects	Traffics to nucleus	
α1-adrenergic	Increases firing (23) Increases [Ca ⁺⁺] _i (33) Increases VP/OT release (23,24) Recruits P2X2/3 and 7 (27)	GIRK P2X				
P2Y-purinergic	Increases [Ca ⁺⁺] _i (34) Increases VP/OT release (36)	TRPV				
NK1-neurokinin	SP increases VP/OT release (47)	GIRK K _{2P}	Gs			
NK3-neurokinin	Senktide increases VP/OT release (49)				-Ligand induced in SON/PVN	
AT1-angiotensin	All increases VP/OT release (57) All potentiates osmotic stimulation of VP/OT release (42)	GIRK TRPV	Gi	Yes	-Neuronal cultures -Alters gene expression	MAPK/ERK
mGluR1	Increases VP/OT release (90)	GIRK K _v (I _A) K _{Ca} (SK)				
Muscarinic	Promotes phasic firing (104)	K _{Ca} K _v (I _A)				
G _i PCR						
GABA _B	Modulates firing (94)	Ca ⁺⁺ _v Activates K _{ir}		Yes		

* Also see (15) for more complete listing of effects and references.