MINIREVIEW

Hormonal and Neurotransmitter Roles for Angiotensin in the Regulation of Central Autonomic Function

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In this review we present the case for both hormonal and neurotransmitter actions of angiotensin II (ANG) in the control of neuronal excitability in a simple neural pathway involved in central autonomic regulation. We will present both single-cell and whole-animal data highlighting hormonal roles for ANG in controlling the excitability of subfornical organ (SFO) neurons. More controversially we will also present the case for a neurotransmitter role for ANG in SFO neurons in controlling the excitability of identified neurons in the paraventricular nucleus (PVN) of the hypothalamus. In this review we highlight the similarities between the actions of ANG on these two populations of neurons in an attempt to emphasize that whether we call such actions "hormonal" or "neurotransmitter" is largely semantic. In fact such definitions only refer to the method of delivery of the chemical messenger, in this case ANG, to its cellular site of action, in this case the AT_1 receptor. We also described in this review some novel concepts that may underlie synthesis, metabolic processing, and co-transmitter actions of ANG in this pathway. We hope that such suggestions may lead ultimately to the development of broader guiding principles to enhance our understanding of the multiplicity of physiological uses for single chemical messengers. [E.B.M. 2001, 226:85–96]

Key words: angiotensin; neurotransmitter; central autonomic control; subfornical organ; paraventricular nucleus

Angiotensin II (ANG) is an 8 amino acid peptide recognized for its endocrine roles in the regulation of vascular resistance and control of aldosterone secretion. In association with the perceived global role of ANG in the regulation of body fluids, many studies carried out over the past 20 years have also identified complementary functions for ANG within the CNS. Circulating ANG has been shown to act at circumventricular organs (CVOs, specialized CNS sites that lack the normal blood brain barrier) to stimulate drinking, vasopressin (VP), oxytocin (OXY), and ACTH secretion, to enhance sympathetic outflow, and to control baroreflex sensitivity. Interestingly, ANG-sensitive neurons located within one of these CVOs apparently use this peptide as a neurotransmitter to communicate with regions of the CNS protected by the blood–brain barrier (1). Identified central actions of ANG are not, however, exclusively associated with the regulation of fluid volume. Substantial evidence has also suggested physiological roles for this peptide in long-term potentiation in the hippocampus (2), the control of reproduction (3, 4), and memory (5, 6).

The paraventricular nucleus (PVN) of the hypothalamus is one such CNS site that has been suggested to receive ANGergic input from the SFO. The PVN is an essential hypothalamic nucleus in the regulation of a number of neuroendocrine and autonomic functions including body fluid homeostasis and cardiovascular regulation. Neurons in the PVN must be able to monitor the body's internal environment, integrate these messages, and send the appropriate signals to both other central nuclei and peripheral regulatory organs so that a constant environment is maintained. The release of the neuropeptides OXY and VP from neurosecretory neurons represents one of the major mechanisms through which the PVN is able exert control over the interior milieu.

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In this review, rather than attempt to summarize and integrate all that is known about potential central actions of ANG in autonomic control (for review see [7–9]) we focus specifically on a single neuronal pathway from subfornical organ (SFO) to PVN (Fig. 1). Here we review data highlighting the roles of ANG both as a circulating chemical messenger (hormone) influencing the excitability of SFO neurons and as a *bona fide* neurotransmitter delivered to its site of action synaptically, and as a consequence controlling the excitability of PVN neurons.

Angiotensin Receptors

A large number of studies have utilized both immunocytochemical and *in situ* hybridization techniques to describe the distribution of ANG receptors within the CNS. The majority of early work identified the autoradiographic distribution of labeled ANG as an indication of ANG-binding sites within the brain (10–12). All of these reports identified high densities of ANG binding in forebrain CVOs, including the SFO and the ME. Additional binding in hypothalamic regions with suggested roles in the regulation of body fluid balance was described including the SON and PVN.

Caudal aggregations of ANG binding were also identified in the dorsal vagal complex, including the nucleus tractus solitarius (NTS) and the most caudal of the CVOs, the area postrema (AP). From these observations emerged the picture of a central ANG system, with a global role in controlling neuronal excitability, which was complementary to the peptide's peripheral role in the regulation of body fluids. The attractiveness of such an hypothesis sustained its popularity despite the emergence of considerable data describing additional binding sites for ANG in hippocampus, locus coeruleus, cerebellum, olfactory tract, superior colliculus, and medial geniculate (10, 11), sites without established roles in the regulation of body fluids. These observations did, however, provide clear indications that ANG may serve multiple roles as a neurotransmitter in neural pathways with distinctly separate functional roles.

The development of non-peptidergic ANG antagonists provided the primary technological impetus for the description of separate ANG receptors ($AT_{1(A\ and\ B)}$ and AT_2), which were eventually shown to have differential distribu-

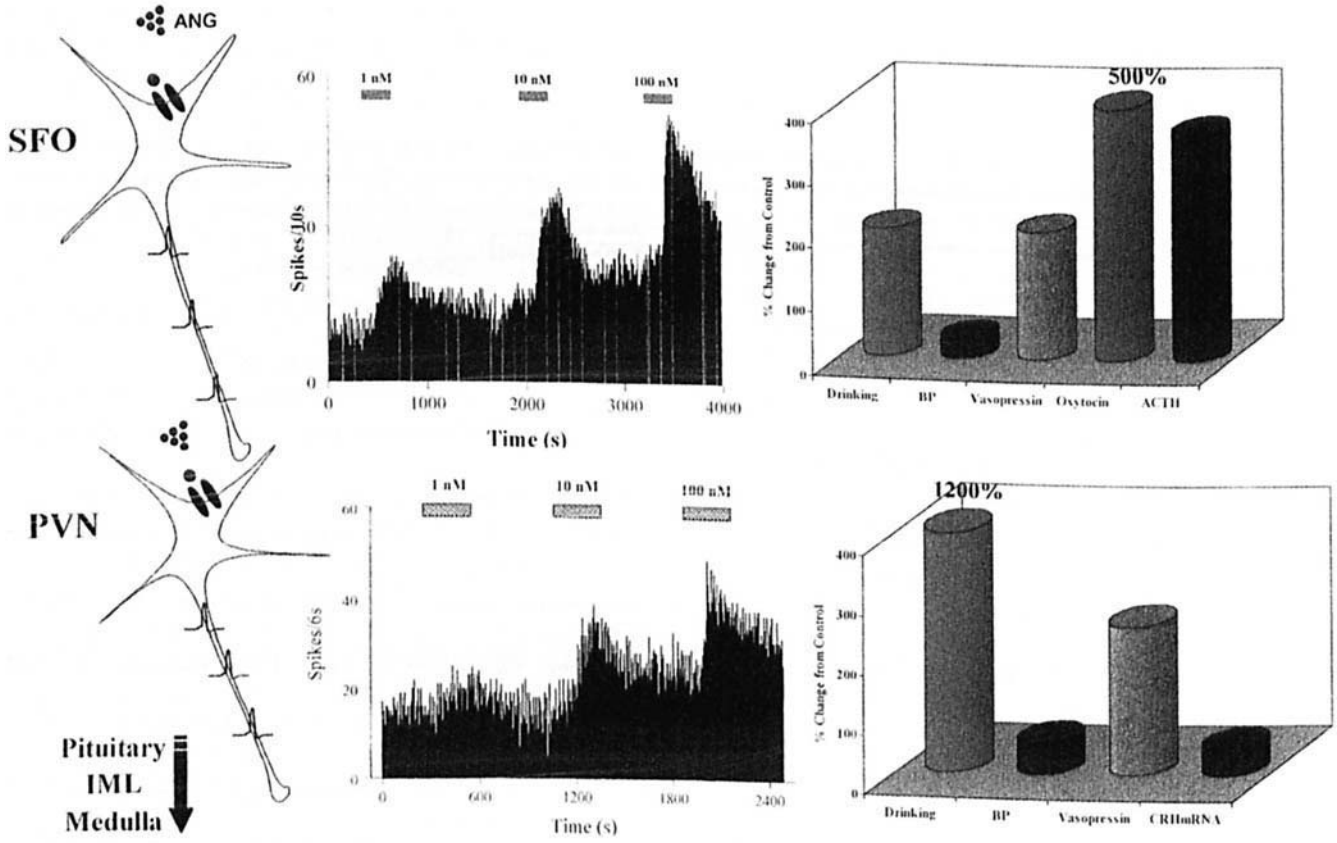

Figure 1. Schematic illustration of cellular and physiological actions of ANG in both SFO and PVN. The schematic representation of an SFO neuron projecting to PVN shown on the left represents the neuronal population that is the focus of this review. The center panels show examples of an SFO and PVN neurons' responses to bath applied ANG *in vitro*. These ratemeter records illustrate dose-dependent excitatory effects of ANG on these neurons that are abolished by AT_1 receptor antagonists (data not shown here). On the right the histograms illustrate schematic representations of the known physiological effects of exogenous ANG administration into SFO (top) and PVN (bottom). For display purposes changes greater than 400% are labeled above the cylinder on these graphs.

tions in the adult brain. Such conclusions were initially drawn from experiments demonstrating differential displacement of ANG binding with AT_1 and AT_2 antagonists (11, 13–15). Interestingly these studies have again suggested some degree of functional homogeneity in that AT_1 receptors predominate in all areas with established roles in the control of body fluids including SFO, organum vasculosum of the lamina terminalis (OVLT), medial preoptic nucleus (MPO), PVN, supraoptic nucleus (SON), dorsal motor nucleus of the vagus (DMV), nucleus tractus solitarius (NTS), area postrema (AP), and nucleus ambiguus. In addition, they have shown varied distribution of both receptor subtypes in the other regions of the brain where ANG binding has been described.

Recently numerous reports using more definitive *in situ* hybridization techniques have effectively confirmed such observations by describing the distribution of mRNAs for AT_{1A}, AT_{1B}, and AT_2 receptors (16–18). Interestingly, one of these reports has also suggested that the AT_{1A} receptor may be preferentially distributed in CNS sites accessed by circulating ANG such as the SFO, while AT_{1B} was found in sites protected by the blood–brain barrier (17). The potential functional relevance of this observation has, however, yet to be addressed.

Angiotensinergic Pathways in the Brain

The data described above demonstrate an extensive distribution of ANG-binding sites at locations within the brain that cannot theoretically be accessed by circulating ANG (this peptide does not cross the normal blood–brain barrier). This information provided the first persuasive evidence for the existence of specific angiotensinergic neurons and thus pathways within the CNS. Such observations led to a tremendous investment of effort directed toward the identification of all components on the renin angiotensin system within the CNS. Again, here we focus on the literature describing such components in the SFO and PVN. Despite this effort, controversy still exists as to whether all components of the system are contained in single "angiotensinergic" neurons.

Angiotensin. Support for the existence of such a network came from the demonstration of nerve cell bodies and fibers that bound immunocytochemical probes directed against ANG (1, 19–23). One of these studies in particular seemed to point to the existence of an interconnected angiotensinergic network of neurons that matched the anatomical description of pathways playing significant roles in the control of fluid balance (1). Thus angiotensinergic neuron identified in the SFO, which send ANG immunoreactive fibers (AIF's) to second-order neurons in the MPO, SON, and PVN. Neurosecretory cell bodies in SON and PVN have in addition been shown to be ANG immunoreactive and again give rise to AIFs which course to both the posterior pituitary (PP) and median eminence (ME). These immunocytochemical studies therefore lead to the conclusion that many nerve cell bodies contain ANG-like immunoreactivity and apparently transport this peptide through identified axonal projections to terminal sites of these cell bodies where the peptide is presumably utilized as a neurotransmitter. Whether these presynaptic neurons in fact contain the biochemical machinery necessary for the synthesis and post-translational processing of this peptide cannot, however, be addressed using such immunocytochemical probes.

Angiotensinogen. There is really no dispute with regard to the conclusion that A_o is widely distributed within the CNS (24–30). The forebrain distribution of A_o reported in these studies is also for the most part as expected in that it overlaps with the regions described demonstrating ANG immunoreactivity including the SFO, PVN, SON, and MPO. The primary controversy with regard to the distribution of A_o lies with the question of whether it is synthesized in glia or neurons. The immunocytochemical data are clear with a number of different reports showing A_o-like immunoreactivity localized in both of these cell types (29, 31). In contrast it would appear that nearly all studies have utilized *in situ* hybridization techniques to describe A_o mRNA distribution have localized this gene product in glial cells (28, 31). This apparently paradoxical lack of colocalization has led to the recent suggestion that glial cells may in fact be responsible for the production of A_o, which is then released and taken up by neurons where it undergoes further processing (32). Although the specific mechanisms underlying such sequences have yet to be described (secretion and uptake of A_o), multicellular processing is clearly a dominant feature of the peripheral renin angiotensin system.

Renin. Although studies have described the presence of renin or renin mRNA within the CNS (33–35), to date there has apparently been no systematic attempt to define the distribution of this enzyme either in specific anatomical regions or in specific cell groups.

Angiotensin Converting Enzyme. The reported profile for the distribution of ACE is again, as one would predict, in accordance with the primary regions of the CNS where ANG has been identified. The SFO as well as the choroid plexus (36–38) has been shown to selectively bind ^{125}I-351A, an inhibitor of this enzyme. Additional binding has been reported in forebrain autonomic centers in which ANG has been localized, including the PVN, SON, MPO, and medial septum (MS) (36–39).

The data presented above therefore confirm the presence of all of the traditional components of the renin angiotensin system within the CNS. This, however, is not the issue when considering whether ANG satisfies the classical criterion that individual neurons must contain all of the machinery for synthesis and secretion of the "putative neurotransmitter." Clearly the available data do not allow ANG to meet this criterion. Although the cellular localization of renin and ACE has not yet been satisfactorily addressed, the majority of evidence regarding A_o suggests that while the peptide itself can be found in neurons the mRNA associated

with synthesis appears to be primarily located in glia. These observations support the conclusion that the production of ANG for use by neurons as a neurotransmitter is a multicellular process in contrast to that for other transynaptic messengers. Perhaps such a conclusion should not be altogether surprising in view of our current understanding of the mechanisms underlying the production of this peptide in the periphery.

Hormonal Effects in Subfornical Organ

Drinking. It was perhaps the initial recognition that circulating ANG stimulated drinking as a direct result of actions in the CNS that did more than any single observation to focus attention on actions of this peptide in the brain. The fact that such effects were observed following both systemic (40, 41) and ICV (42, 43) administration of ANG, when combined with the presence of ANG receptors in the CVOs, served to focus attention on these specialized structures as potential targets at which ANG influenced neural tissue to elicit such effects. The microinjection studies of Simpson *et al.* (44) suggested SFO as the target for circulating ANG (Fig. 1), an hypothesis that was eventually confirmed by the observation that destruction of the SFO abolished the drinking response to systemic ANG (45–47). Many different groups have also examined the potential role of projections emanating from the SFO to hypothalamic regions implicated in the control of drinking (41, 47, 48). The technical inability to obtain single-unit recordings from conscious freely moving animals has, however, precluded a more in-depth analysis of the role on central ANG in controlling the excitability of neurons *directly* involved in the control of fluid intake.

Hormone Secretion. ANG has been clearly shown to play a double role, both as hormone and neurotransmitter, in the control of both VP and OXY from the posterior pituitary (Fig. 1). Increases in circulating ANG have been shown to result in large increases in the concentration of both OXY and VP, effects that are abolished by destruction of the SFO (49, 50). In addition, both systemic (51) and local (52) application of ANG have also been shown to result in AT_1 receptor-mediated increases in neuronal activity of antidromically identified OXY and VP neurons. Similar data have been obtained demonstrating roles for both peripheral (53, 54) and central (55) ANG in the control of ACTH secretion from the anterior pituitary presumably through the same population of SFO neurons and their projections to the PVN (Fig. 1). Further evidence in support of such a central regulatory role for ANG in control of the adrenocortical axis comes from the demonstration of profound effects of glucocorticoids in regulation of angiotensinogen mRNA expression in diverse regions of the CNS including the PVN (56).

Cardiovascular Control. The initial observations of Bickerton and Buckley (57) identifying a centrally mediated component of the hypertensive response to systemic ANG represent the first of many studies demonstrating central roles for both circulating and centrally produced ANG in the regulation of cardiovascular function. Following identification of the CVOs as central targets for circulating ANG it quickly became apparent that the SFO represented a site at which ANG could exert profound influences over central cardiovascular control. Lesion (45, 46) and microinjection (58) studies supported the conclusion that the central pressor effects of circulating ANG resulted from its ability to influence the activity of SFO neurons. This view has since been further corroborated by both *in vivo* (59–63) and *in vitro* (64–67) demonstrations of excitatory effects of ANG on SFO neurons (Fig. 1). Systemic ANG has also been reported to increase *c-fos* expression in SFO neurons (68).

Neurotransmitter Effects in Paraventricular Nucleus

In addition to the traditional endocrine roles of circulating ANG, there is considerable evidence supporting a nonclassical function as a *bona fide* neurotransmitter. This concept is derived from observations that seem to fulfill the previously established criteria for implication as a neurotransmitter, including the identification of ANG-like immunoreactivity in somata, fibers, and nerve terminals, as well as the appropriate localization of post-synaptic receptors (Fig. 2). Historically, it has been maintained that intracellular and even specific subcellular (pre-synaptic terminal) localization of the materials required for direct synthesis of the putative neurotransmitter molecule is an absolute prerequisite. In the case of ANG, this direct connection does not seem to hold absolutely. Rather than discount the potential for true ANG-mediated neurotransmission, we will discuss other alternatives that may justify amendments to these previously outlined criteria.

Traditional Neurotransmitter Criteria. There is now considerable evidence of functional synaptic release of ANG in PVN. Push–pull perfusion experiments have demonstrated endogenous physiologically relevant release of ANG in the PVN (69, 70). The physiological (71) and electrophysiological (72–75) consequences of activation of putative ANGergic input to PVN neurons have also been shown to be abolished by specific AT_1 receptor antagonists. Finally exogenous administration of ANG and ANG-related peptides into this region also mimics the effects of electrical or chemical stimulation of presumed ANGergic cell groups in SFO (42, 76). Despite such persuasive evidence it needs to be clearly stated that ANG does not satisfy one of the standard criteria necessary to be accepted as a traditional neurotransmitter, i.e., the synthetic machinery necessary for its production has not been localized in nerve terminals of SFO neurons in the PVN. However, this situation is similar for all neuropeptides that have been primarily suggested to be synthesized in the cell body of the peptidergic neuron and then transported to axon terminals for exocytotic release following action potential triggered calcium influx. In the case of ANGergic SFO neurons this modus operandum is

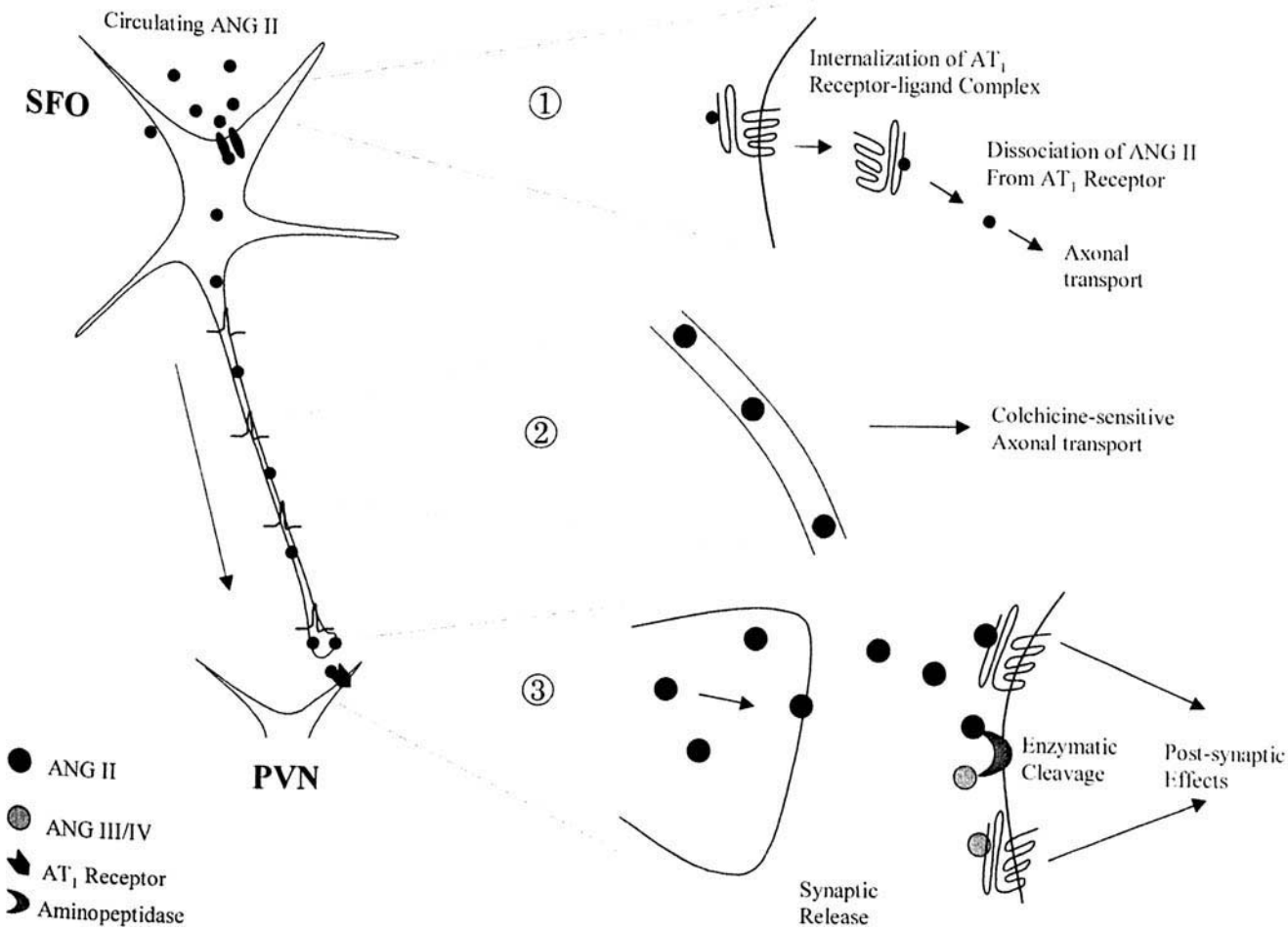

Figure 2. Schematic representation of angiotensinergic neurotransmission from the SFO to PVN. Circulating ANG binds to AT_1 receptors on neurons in the SFO (①) and is internalized as the ANG–AT_1 receptor–ligand complex. Following dissociation from the AT_1 receptor, ANG may be anterogradely transported toward the presynaptic terminal via a colchicine-sensitive mechanism, as shown in (②). (③) Synaptic release of vesicular ANG occurs with action potential propagation to the presynaptic terminal. In the synaptic cleft, ANG II may bind to AT_1 receptors on the post-synaptic PVN cell or may be cleaved to ANG III by aminopeptidases present in the cleft. Subsequently, ANG III may also act on post-synaptic AT_1 receptors.

clearly supported by studies describing ANG immunoreactivity in somata which could be visualized more clearly following pretreatment with colchicine, indicating that a large amount of ANG peptide is of somatic origin, and is transported in an anterograde fashion toward axon terminals, potentially for release during chemical neurotransmission (1).

Together with the anatomical and neurochemical evidence suggesting potential for the use of ANG as a neurotransmitter, many electrophysiological studies have shown direct evidence in support of this hypothesis. PVN neurons projecting to the spinal cord receive excitatory, ANGergic input from the SFO (72), as do identified SON neurons (77). Interestingly, in these *in vivo* electrophysiological studies, electrical stimulation of the SFO resulted in a long-duration post-synaptic excitation. Following treatment with a non-peptidergic AT_1 receptor antagonist, this long-duration response was blocked, with only the underlying rapid excitatory phase remaining. These data suggest that chemical neurotransmission at this SFO–PVN synapse is mediated by both a rapid (putative amino acid) and slow (ANG) messenger. *In vitro* studies have shown that ANG can have direct, excitatory effects on neurons within the PVN (Fig. 1) that are blocked by AT_1 receptor antagonists (67, 75). In addition to direct electrophysiological measurements, evidence suggests that many of the physiological responses governed by the SFO–PVN pathway are mediated by neurotransmitter actions of ANG. Direct microinjection of ANG into the PVN causes dose-dependent elevations in blood pressure (73, 78), drinking (78), CRH mRNA (79), and vasopressin release (80) (Fig. 1). Conversely, receptor blockade can prevent similar effects initiated by stimulation of the SFO-PVN pathway, thus providing evidence for inhibition of the effects of endogenous ANG release. The hypertensive effects of SFO stimulation are inhibited by blockade of AT_1 receptors by losartan (81). Similarly, the dipsogenic response to SFO stimulation can be antagonized by local application of saralasin to the MnPO (48), suggesting that ANGergic pathways from the SFO also underlie this behavior.

Novel Concepts in Angiotensin Neurotransmission. Although ANG II has been localized in cell bodies and fibers, and exogenous administration can exert specific physiological effects, there is some controversy regarding which form of ANG actually mediates post-synaptic responses within the CNS. Over the course of the last decade, there has been increasing evidence suggesting that ANG III has direct biological effects in a number of central autonomic systems (82, 83), apparently through actions at AT_1 receptors (84). These data come largely from studies employing exogenous administration of ANG III. More recently, however, the development of more selective aminopeptidase m and p inhibitors has allowed investigators to elucidate potential roles of endogenously produced ANG III. These studies have been enlightening, and they challenge some of the dogma surrounding central actions of ANG peptides. Firstly, in many central ANGergic pathways aminopeptidase m has been localized (85) and has shown to be increased in brains of spontaneously hypertensive rats (86). Secondly, inhibition of these enzymes activity leads to a reduction in systemic blood pressure (87) and VP secretion (88), presumably through a decrease in ANG III production. Note that these two effects are normally ascribed to the actions of ANG II in the large body of literature on the subject. Clearly, these studies serve to fulfil two of the traditional criteria for identification of ANG III as a neurotransmitter: (i) exogenous administration induces biological effects and (ii) blockade of action of endogenous ANG III (in this case by blocking cleavage of ANG II to ANG III). The observations that inhibition of aminopeptidase activity largely attenuates hypothalamic actions normally ascribed to ANG II (Fig. 2) suggest two key features of this system which must be paramount to the ANGergic neurotransmitter system: (i) that conversion of synaptically released ANG II to ANG III must occur rapidly and (ii) that ANG III must act on the same receptor population that ANG II is believed to act on but with at least equal or higher affinity. Should this second feature not hold true, it would be expected that blockade of aminopeptidase activity would not have significant biological effects, as the overspill of ANG II would be able to activate AT_1 receptors to a similar degree, thus producing the desired biological effect, be it an increase in blood pressure or VP release.

One of the traditional neurotransmitter criteria outlined previously is that of location of machinery for intrinsic production of the transmitter molecule. Typically, this consists of one of the final enzymes required for synthesis and is often used to identify the neuronal chemical phenotype. This criterion may not, however, hold true with ANG neurons, as there is a considerable discrepancy in the literature with respect to the colocalization of ANG II and angiotensin-converting enzyme and/or angiotensinogen as outlined above. This apparent shortfall may be resolved with the unique coincidence that many angiotensinergic neurons are also angiotensin sensitive. As with other G-protein coupled receptors (GPCRs), AT_1 receptors internalize along with their ligand as a form of homologous desensitization (89). Subsequent to internalization, the receptor–ligand complex dissociates, leaving free ANG II within the cytoplasm. Although this process has not been studied directly in ANGergic neurons, circumstantial evidence suggests that somatically originating ANG is transported out of the soma. This may provide a potential mechanism through which ANGergic neurons may effectively acquire their transmitter molecule through an energetically favorable mechanism that is highly efficient, although not traditional.

Cellular Mechanisms of Action

Effects on Signal Transduction Pathways. The distribution and ontogeny of AT receptors have been extensively studied in the CNS, and it is clear that receptor subtype is critical in the action of ANG in any tissue. Biochemical studies have only recently begun to uncover the multitude of signal transduction pathways that these receptors are capable of activating. In addition, studies have begun to describe the ramifications of such actions to physiological and pathological processes.

While the signal transduction cascades which underlie the physiological effects of ANG remain largely unidentified, a number of reports have documented the relationship between ANG and G protein coupled receptors (Fig. 3). Studies reveal that AT_1 receptors are coupled to either activation of phospholipase C (PLC) and the subsequent stimulation of phosphotidyl inositol (PI) hydrolysis (90–92) or to the inhibition of adenylyl cyclase (91). PI hydrolysis effects are largely dependent upon mobilization of internal calcium and/or activation of protein kinase C (PKC). Studies have also indicated AT_1 receptor stimulation of phospholipase D (PID) and phospholipase A2 (PLA2), in addition to the Ras/Raf/MAP kinase and JAK (93–95). The physiological consequences of activation of these pathways by ANG, however, remain poorly understood. In addition, stimulation of AT_1 receptor also leads to increased transcription of immediate early genes such as *c-fos, fos b, c-jun, jun b,* and KROX-24 and to the production of their respective gene products in the SFO, MnPO, PVN, and SON (90, 96).

Most of the information on the effects of AT_2 receptor activation (Fig. 3) on neurons has been obtained from either tumor cell lines or from the newborn rat hypothalamus in culture. While AT_2 receptors are also G protein coupled, experiments in PC12 cells suggest that their actions are largely dependent upon activation of PLA2 and the subsequent production of arachidonic acid (AA) (93, 97–99). Stimulation of phosphotyrosine phosphatase (i.e., MKP-1) and/or serine/threonine phosphatase type 2A (PP-2A) in cultured neurons has also been observed, which may underlie the neurite outgrowth that has been reported (93, 100, 101). Sumners *et al.* have shown in neonatal neurons that AT_2 receptor effects are in part dependent on a decrease in cGMP, an end point that may be the critical element in many of this receptor's actions (102).

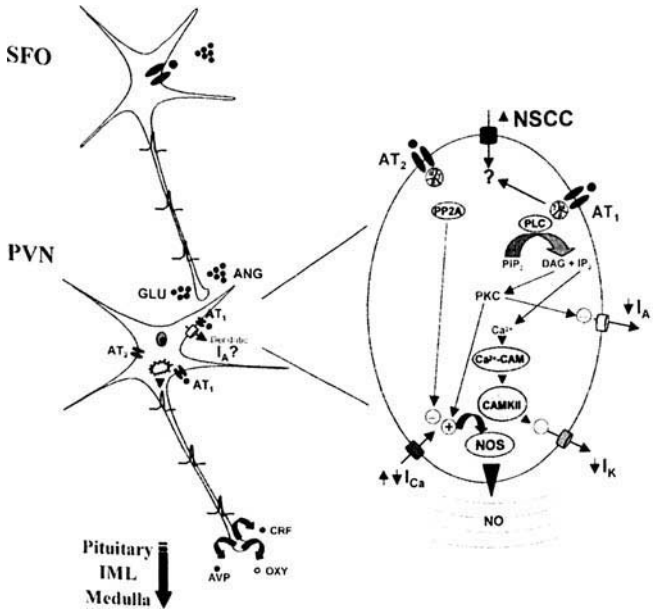

Figure 3. ANG activates signal transduction cascades that result in neuronal excitation and neurotransmitter release. This schematic summarizes the intracellular signaling pathways involved in mediating ANG excitation of PVN neurons. Comprehensive details regarding ANG facilitation and inhibition of I_A, I_K, I_{Ca}, and NSCC are described in the text. AT_1 receptor mediated effects are G-protein coupled and are largely dependent upon activation of PLC and the subsequent mobilization of internal calcium and stimulation of PKC. PKC is capable of phosphorylating ion channels directly and modulating their activity while an increase in internal $[Ca^{2+}]$ results in either CAMKII and/or NOS activation. Somatic excitation following ANG receptor activation is principally due to inhibition of I_A and I_K coupled with increases in I_{Ca} and NSCC which ultimately result in a decrease in net outward current as previously described. AT_2 receptor activation is capable of modulating hypothalamic ion channels (e.g., I_{Ca}) although the AT_2 receptor contribution to neuronal excitation is considered minimal. Dendritic ion channels, specifically I_A, are also regulated by ANG. Inhibition of dendritic I_A via AT_1 receptor activation maximizes incoming excitatory stimuli, resulting in somatic depolarization. The net effect of ANG–AT_1 receptor binding is an increase in PVN "output" and thus facilitation of neurotransmitter release at the neuron terminal, which results in the physiological responses, observed following PVN stimulation. Arrows represent pathways. ANG, angiotensin II; GLU, glutamate; I_A, transient outward potassium current; I_K voltage-dependent delayed rectifier; NSCC, nonspecific cationic conductance; I_{Ca}, total calcium current; PLC, phospholipase C; PiP_2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; IP_3, inositol 1,4,5-triphosphate; NOS, nitric oxide synthase; NO, nitric oxide; PKC, protein kinase C; CAMKII, calcium/calmodulin-dependent protein kinase II; PP2S, serine/threonine phosphatase type 2A.

Effects on Ion Channels. The evolution of sophisticated patch clamp techniques to measure the effects of ANG on membrane-located ion channels has revealed many of the mechanisms by which this peptide modulates neuronal activity. ANG has been shown to act upon a variety of ionic conductances, including voltage dependent potassium and calcium currents as well as nonselective cationic currents. Ultimately, the effects of ANG on these channels are largely dependent upon the type of angiotensin receptor localized in a particular cell type. In many cases, AT_1 and AT_2 receptor activation has been observed to elicit opposing outcomes on similar channels. Figure 3 shows possible outcomes of ANG binding on AT_1 and AT_2 receptors to elicit excitatory effects on a PVN neuron. Obviously, the final physiological actions of this peptide represent the cumulative effects of AT receptor-mediated actions on multiple ion channels, and ultimately on the complex interactions between these channels in single neurons.

Potassium currents. ANG has been shown to act on both the transient outward (I_A) and the delayed rectifier (I_K) potassium currents. I_A is thought to exist to some degree in most central neurons is thought to play a critical role in regulating action potential timing and propagation of dendritic inputs. I_A can be identified by its unique voltage dependent activation, fast kinetics of inactivation, and pharmacological sensitivity to millimolar concentrations of 4-aminopyridine (4-AP) (103). In neurons, I_A dampens excitation and increases the interspike interval and is important in the maintenance of synaptic input (103). Correspondingly, I_A is extremely important in the regulation of neuronal burst firing, a critical phenomenon in modulating neuropeptide release from PVN and SON neurons (104, 105). In SFO neurons, ANG has been shown to decrease I_A as a result of interactions with AT_1 receptors, an observation consistent with the finding that ANG caused an AT_1 receptor-mediated decrease in open probability of single A-type K^+ channels (74, 93, 106). The AT_1-mediated reduction in I_A has been observed in neurons in the SFO, SON, and PVN, where such effects are maintained in synaptic isolation (TTX or Ca^{2+}-free/Co^{2+} medium). These data may explain the underlying excitatory actions of ANG on these neuronal subpopulations, specifically the observed increase in firing frequency. They do not, however, correlate well with the reported decreased input resistance in PVN and SON neurons following ANG application, suggesting an increase rather than a decrease in conductance (74, 75, 107).

The specific K^+ channel isoforms underlying the observed I_A in these neurons have not yet been elucidated. The cloned K^+ channels from rat brain, Kv1.4 and Kv3.4, have, however, been demonstrated to give rise to A-type currents (106). The effects of AT_2 receptor stimulation on I_A have not been studied as extensively, although a potentiation of I_A has been reported (108).

The intracellular signaling pathways, which underlie the modulatory effects on I_A, remain less well understood. Preliminary studies indicate that PKC may be involved as the PKC activator PMA dramatically reduces the open time probability of the A-type channel (90, 109). AT_2 receptor modulation of I_A appears to be dependent on AA and PLA2 as the response of ANG on I_A via the AT_2 receptor can be largely abolished via inhibition of PLA2 (93).

The effects of ANG on the voltage-dependent delayed rectifier, I_K, a current critical in neuronal repolarization, have also been studied. I_K channels are much more diverse than their I_A counterparts, but again they can be differentiated by their biophysical and pharmacological characteristics. The inhibitory nature of the action of ANG on I_K correlates well with the excitatory actions of ANG in SON and cultured brainstem/hypothalamic neurons. Sumners *et*

al. demonstrate that in newborn hypothalamic and brain stem cultures ANG reduces I_K, an effect that is mediated by the AT_1 receptor (110). This reduction is partially abolished by inhibitors of either calmodulin and/or CaM KII or was mimicked by intracellular application of activated (autothiophosphorylated) CaM KIIα. Correspondingly, ANG actions at the AT_1 receptor also stimulate an increase in neuronal intracellular calcium and consequently CaM KII activity. Concurrent inhibition of PKCα, and CaM KIIα completely abolished the ANG effects on I_K, demonstrating the involvement of PKCα. Interestingly, single-cell RT-PCR analysis has revealed the presence of AT_1 receptor, CaM KIIα, and PKCα subunit mRNAs in those neurons that show a decrease in I_K following ANG application (111). Thus inhibition of I_K involves a Ca^{2+}/calmodulin/CaM KII signaling pathway as well as PKC (93). Contrary to findings in hypothalamic cultures, Li and Ferguson show that I_K is not reduced by ANG in PVN neurons and therefore does not likely mediate ANG-induced excitatory actions in this nucleus (75).

Confoundingly, activation of the AT_2 receptor results in a potentiation of I_K that can be mimicked by CGP42112A (AT_2 receptor agonist) (93). The AT_2 receptor-mediated stimulatory effects of ANG are abolished by pertussis toxin, suggesting the involvement of a G_i or G_o protein (108). PP-2A has been implicated as okadaic acid and anti-PP2A antibodies abolish the AT_2 receptor augmentation of I_K. Furthermore, intracellular application of $G_{i\alpha}$ antibodies abolishes the response. In this regard, AT_1 receptor activation and the subsequent inhibition of both I_A and I_K likely comprise major mechanisms that underlie known physiological consequences of ANG receptor activation in both SFO and PVN.

Calcium currents. Calcium channels, ubiquitous among excitable cells, are known to play important regulatory and electrical roles. Increases in intracellular calcium have been shown to have a myriad of consequences, including activation of Ca^{2+}-dependent enzymes, support of mitochondrial function, and modulation of other ion channels, such as Ca^{2+}-activated K^+ channels. These channels, much like K^+ channels, can be differentiated on the basis of their biophysical properties and their sensitivity to pharmacological agents. ANG has been shown to act on several calcium channel subtypes to increase neuronal excitability (Fig. 3).

Sumners *et al.* demonstrate that ANG increases total calcium current, I_{Ca}, in response to AT_1 receptor activation (97). In contrast, AT_2 receptor stimulation does not appear to have an effect on I_{Ca} in cultured neurons (93, 97, 110, 112). Interestingly, the ANG-induced modulation of calcium channels appears to be tissue specific, as ANG has been demonstrated to have opposite effects on similar channel subtypes in different tissues. Shapiro *et al.* demonstrate that ANG inhibits N-type Ca^{2+} channels in rat superior cervical ganglion cells. This inhibition is blocked by losartan, attenuated by the inclusion of GDP-β-S in the pipette, is pertussis toxin insensitive and voltage independent. BAPTA, a calcium chelator, attenuates this inhibition suggesting a dependence on mobilization of intracellular calcium (113). In contrast, enhancement of N-type Ca^{2+} current subsequent to AT_1 receptor activation was observed in rat sympathetic neurons (102). T-type Ca^{2+} channels critical in excitation contraction coupling and facilitation of neurotransmitter release at the soma are inhibited by ANG in the undifferentiated NG108-15 neuroblastoma × glioma cells (93, 114). The actions of ANG on the L-type Ca^{2+} channel appear to be paradoxically 2-fold in rat retinal ganglion neurons, where interestingly ANG inhibits L-type Ca^{2+} currents in one population of cells while potentiating them in another (115). Unfortunately, information investigating the pathways that underlie this dichotomy of angiotensinergic effects on L-type Ca^{2+} channels remains inconclusive. AT_1 modulation does, however, appear to occur through an indirect signaling pathway rather than being membrane delimited. The stimulation of I_{Ca} largely appears to be similar in nature to the reduction of I_K as it involves G_q protein cascade and PLC, although it is documented that CAM kinase II is not involved (93). In nondifferentiated NG108-15 neuroblastoma × glioma cells, AT_2 receptor activation decreases T-type Ca^{2+} currents via activation of phosphotyrosine phosphatase rather than PP-2A (93, 114). Clearly, ANG is capable of modulating Ca^{2+} channels in a number of systems that could potentially underlie neuronal activation. In the hypothalamus, a number of studies have suggested a decrease in net outward current following ANG receptor stimulation, in part mediated by an increase in calcium conductance, is responsible for neuronal excitation. The calcium channel subtypes responsible for these observations have not yet been identified.

Nonselective cationic conductance. The nonselective cationic conductance (NSCC), a voltage independent current, has recently been implicated in facilitating the depolarizing effects of a number of well-known hormones including ANG (116). Although, not as extensively studied as the K^+ and Ca^{2+} currents, the NSCC has been shown to underlie the excitatory effects of ANG on MnPO and SON neurons (117, 118). The actions of ANG on NSCC are AT_1 receptor mediated as they are abolished in losartan. The signal transduction pathways that trigger the activation of NSCC following AT_1 receptor stimulation remain unknown (102, 118). While a number of studies suggest the involvement of a NSCC based on the observed decrease in input resistance (increase in cationic conductance) and reversal potential of the depolarizing current, few studies have investigated the NSCC and ANG using voltage clamp techniques. Chakfe and Bourque have observed that ANG modulates a stretch-inactivated cation channel that also underlies the intrinsic osmosensitivity of magnocellular SON neurons (116), suggesting an intriguing site for further integration of diverse peripheral signals describing body fluid status.

Nonsomatic Effects of ANG

Somatic actions of ANG have been assumed to be primarily responsible for the demonstrated effects of this peptide on neuronal excitability and subsequent release of neuropeptide. Important novel roles for ANG have more recently been suggested in the modulation of terminal and dendritic ion channels as well as the regulation of synaptic efficacy.

Effects on Dendrites. Dendritic ion channels are known to have critical roles in the maintenance of synaptic efficacy and generation of neuronal excitation. Although ANG has been shown to have excitatory actions on a number of neuronal systems, the effects of this peptide on dendritic ion channels remain unclear. Electrophysiological studies have demonstrated the presence of I_A on the dendrites of neurons in a number of systems, including the hippocampus and cerebellum (119, 120). Further, a number of studies have clearly demonstrated that magnocellular neurons of the SON and PVN express a profound I_A (75, 121, 122). The presence of I_A on the dendrites of magnocellular neurons in PVN and SON has been suggested by the demonstration that magnocellular neurons of the SON, following dissociation and the consequent loss of their dendritic trees, appear to lose their I_A (123). As discussed above, ANG has been shown to inhibit I_A in SON and PVN, an effect that may thus be the result of dendritic rather than somatic actions of this peptide (75, 124).

Co-localization in Terminals. Interactions between GABA and ANG at the nerve terminal have also been suggested by work showing numerous terminals in central portions of the SFO that contain both GABA and ANG immunoreactivity (125). Interestingly, immunogold–silver aggregates recognizing ANG were often detected near nonsynaptic portions of the plasma membrane while GABA immunoperoxidase labeling was most intensely localized to membranes of small clear vesicles that were aggregated near the presynaptic junction. Chan and Pickel postulated that neuronal ANG might modulate the inhibitory postsynaptic responses to GABA following release from single axon terminals (125).

ANG may also modulate glutamate-evoked PSPs as suggested by the work of Xiong and Marshall showing that iontophoretically applied ANG reduces the depolarization induced by iontophoretically applied L-glutamate in locus coeruleus neurons (126). They also report, using intracellular recordings in an *in vitro* brain slice preparation, that ANG depressed non-NMDA mediated EPSPs, demonstrated to be AT_2 receptor specific (blocked by PD123177). Interestingly, the effects of ANG on these neurons are selective for glutamate, as they observe no effects of ANG on the electrical properties of these neurons. They postulate that the effect is likely mediated by an intracellular messenger activated by ANG, and the effects appear to be postsynaptic in nature as they are maintained in low Ca^{2+}/high Mg^{2+} medium (127).

Similarly, co-transmitter interactions between glutamate and ANG have been suggested in PVN by data demonstrating that separate components of the post-synaptic effects of SFO inputs on PVN neurons are the result of glutamate and ANG actions on the post-synaptic cell (126). Intriguingly, such interactions have also been suggested not only to be responsible for the excitation of PVN neurons but also to activate local feedback circuitry that modulates network activity. We have recently reported that ANG, following its excitatory actions on magnocellular neurons in PVN, is capable of increasing the frequency of IPSPs in the same population of cells (128). This increase in IPSPs observed in the magnocellular neurons is shown to be NO dependent, possibly reflecting the interaction of NO and perinuclear inhibitory neurons surrounding PVN. This negative feedback pathway represents an important circuit whereby the regulation of neuronal excitability and thus the extent of neuropeptide release can be tightly controlled.

Conclusions

In this review we have presented the case for both hormonal and neurotransmitter actions of ANG in the control of neuronal excitability in a simple pathway involved in central autonomic regulation. The majority of readers will probably be quite comfortable with the conclusion that ANG exerts hormonal control over the excitability of SFO neurons and perhaps equally uncomfortable with the description of this peptide as a neurotransmitter controlling the excitability of PVN neurons. In this review we have highlighted the similarities between the actions of ANG on these two populations of neurons in an attempt to emphasize that whether we call such actions "hormonal" or "neurotransmitter" is largely semantic. In fact such definitions only refer to the method of delivery of the chemical messenger, in this case ANG, to its cellular site of action, in this case the AT_1 receptor. We have also emphasized in this review some novel concepts that may underlie synthesis, metabolic processing, and co-transmitter actions of ANG in this pathway. We hope that such suggestions may lead ultimately to the development of broader guiding principles to enhance our understanding of the multiplicity of physiological uses for single chemical messengers.

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