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The intricacies of the renin angiotensin system in metabolic regulation

Erin Bruce¹ and Annette D. de Kloet²

¹Department of Pharmacodynamics, College of Pharmacy, University of Florida

²Department of Physiology and Functional Genomics, College of Medicine, University of Florida

Abstract

Over recent years, the renin-angiotensin-system (RAS), which is best-known as an endocrine system with established roles in hydromineral balance and blood pressure control, has emerged as a fundamental regulator of many additional physiological and pathophysiological processes. In this manuscript, we celebrate and honor Randall Sakai's commitment to his trainees, as well as his contribution to science. Scientifically, Randall made many notable contributions to the recognition of the RAS's roles in brain and behavior. His interests, in this regard, ranged from its traditionally-accepted roles in hydromineral balance, to its less-appreciated functions in stress responses and energy metabolism. Here we review the current understanding of the role of the RAS in the regulation of metabolism. In particular, the opposing actions of the RAS within adipose tissue vs. its actions within the brain are discussed.

Keywords

angiotensinogen; obesity; angiotensin-converting enzyme 2; white adipose tissue; food intake

1. Introduction

Obesity is a critical public health concern and many factors contribute to its etiology. One such factor is the renin-angiotensin-system (RAS), the activity of which becomes elevated in obesity [1–5] – an occurrence that is thought to contribute to obesity-related cardiovascular impairments [2, 3, 6–9]. In addition to serving a long-appreciated role in cardiovascular function, the RAS has recently emerged as a key regulator of energy balance. Several studies have examined the impact of activating or inhibiting various components of the RAS on the central nervous system and peripheral control of energy homeostasis [10–21]. These studies have begun to elucidate not only the impact of angiotensin-II (Ang-II) at its type-1 receptor (AT1R) in this regard, but have also determined a potential role for the less-characterized angiotensin type-2 receptor (AT2R) and the putative counterregulatory angiotensin converting enzyme 2 (ACE2)/angiotensin-(1-7) [Ang-(1-7)]/ Mas receptor (MasR) limb of the RAS [22–26]. Together, the results of these previous studies insinuate that targeting

certain components of the RAS may ultimately be revealed as routes for therapeutic intervention for obesity and its co-morbidities. These studies also highlight the complexity of the RAS's involvement in the regulation of energy metabolism.

When considering Ang-II actions at AT1R, per se, there are several lines of evidence indicating that the peptide's direct actions at adipose tissue are trophic and lead to increased fat accumulation [12, 27]. Conversely, when elevated levels of Ang-II impact brain AT1R, negative energy balance ensues, and this is manifested both by decreases in food intake and by increases in energy expenditure [10, 20, 21, 28, 29]. In general, these Ang-II actions, coupled with the propensity of the circulating levels of the peptide to correlate with the level of adiposity, much resemble the actions insulin and leptin, two well-known 'adiposity signals' [30, 31]. As a consequence, it has previously been proposed that Ang-II acts as a novel 'adiposity signal' [9], and the associated line of research that we have conducted was initiated in close collaboration with Professor Randall Sakai.

Over the past several years, we and others have reviewed the literature that supports the proposition that opposing brain vs. peripheral actions of RAS contribute to the regulation of energy balance [9, 32]. In the present manuscript, we similarly discuss studies that support such functions of the RAS, however we now add to the complexity by considering the impact of the ACE2/Ang-(1-7)/MasR axis on metabolism and further considering a role for AT2R in these processes. In regards to the brain actions of the RAS, the focus here is primarily on the neural circuitry that may be involved, and we therefore include new data that assist in elucidating the relevant circuitry. Furthermore, we will examine the seemingly counter-regulatory effects of Ang-II in adipose tissue.

2. The Renin Angiotensin System

Traditionally, the RAS is known as an endocrine system that is critical for the regulation of hydromineral balance and cardiovascular function. Angiotensinogen (AGT), arising primarily from the liver, is cleaved first by kidney-derived renin and then by lung-derived angiotensin-converting enzyme (ACE) to form Ang-II. This octapeptide then acts at its G-protein coupled receptors to exert its physiological and pathophysiological effects.

In humans, angiotensin receptors can be subdivided into two types: the AT1R and the AT2R. Both of these receptors are G-protein-coupled receptors [33–41], the activation of which triggers a number of intracellular signal transduction pathways, such as G-protein-mediated (Gq and Gi), JAK/STAT, and MAPK or ERK intracellular signaling cascades [42, 43] and many of the classical roles of the RAS have been attributed to the activation of one or more of these signaling cascades [44]. In rodents, the AT1R subtype can be further subdivided into the angiotensin type-1a receptor (AT1aR) and type-1b receptor (AT1bR) whose genes are present on separate chromosomes, but are highly homologous [45, 46]. Within the brain, AT1R is primarily present in its AT1aR form; however, in some cases, AT1bR has been detected in brain and it is also abundant in the anterior pituitary and adrenal cortex [33, 47, 48]. AT1Rs are then the target of most of the known physiologic actions of Ang-II, such as vasoconstriction and the induction of sodium and water consumption.

While it is apparent that this ‘traditional’ RAS is involved in energy metabolism, it is also now evident that there exist numerous complexities of the RAS (Figure 1) that must be appreciated when deliberating manipulating the RAS as a therapeutic for obesity and related co-morbidities. For example, the putative counter-regulatory limbs of the RAS – the ACE2/Ang-(1-7)/MasR axis and the AT2R – may similarly serve as routes of obesity intervention [22, 23, 25, 26]. Targeting the AT1R impacts elements of these alternative limbs and it is possible that some of the beneficial actions of AT1R antagonism using angiotensin receptor blockers (ARBs) are mediated by these ‘protective’ limbs of the RAS [49, 50]. It is now also clear that all of the components necessary for Ang-II generation and action exist within numerous tissues including, for example, adipose tissue, brain, skeletal muscle and the gastrointestinal tract and can act in a paracrine/autocrine fashion in these areas [9, 12, 51–59]. The RAS can then have diverse actions in regards to energy metabolism, depending on the tissue that it acts within [9]. While we focus here only on the white adipose tissue and brain actions of the RAS, it is evident that these other tissue RASs may similarly be involved in the regulation of energy metabolism [9, 52–57].

3. The Renin Angiotensin System and the Peripheral Regulation of Energy Balance – RAS in White Adipose Tissue

White adipose tissue (WAT) has long-been understood to serve as a storage site for excess energy in the body. When energy accumulation exceeds energy expenditure over long periods of time, metabolic syndrome and obesity are often the consequence. More recently, it has become apparent that WAT is not only important for energy exchange; it also serves as an endocrine organ secreting ‘adipokines’, which affect food intake, thermogenesis, glucose homeostasis, inflammation and many other functions [60]. As mentioned above, WAT also expresses all of the components of the RAS, such that the effector peptides of the system may be produced within the tissue [23, 61–70]. These adipose-derived RAS peptides may then stimulate receptors localized to adipose tissue itself, or may be released into circulation to stimulate those localized to distant tissues. Comparable to many adipokines, the levels of AGT and Ang-II within the plasma, are positively correlated with the level of adiposity and are regulated by energy status and dietary components, such as glucose and free fatty acids [4, 5, 12, 51, 71–77].

3.1. Angiotensin II Activation of the AT1R and AT2R in White Adipose Tissue

Multiple components of the RAS directly impact adipocyte physiology, and genetic animal models have provided substantial insight into the mechanisms of these effects. In general, these studies suggest that Ang-II within WAT acts to facilitate energy storage. For example, transgenic upregulation of AGT within adipose tissue of mice leads to increased adiposity [12, 24], while its deletion specifically from adipocytes does not affect bodyweight or adiposity but is associated with decreased adipose tissue inflammation, increased metabolic activity, improved glucose tolerance and a reduced propensity to develop obesity-related hypertension [78, 79]. The implication is that increased AGT levels within adipose tissue are sufficient promote adipose expansion and that they are necessary for obesity-related adipose tissue inflammation, as well as glucose intolerance and hypertension. In regards to the Ang-II receptor subtype that may be involved, mice that lack the AT1R throughout the body are

resistant to diet induced obesity, exhibit a reduction in adipocyte size and do not display alterations in adipocyte differentiation, suggestive of a role for the AT1R in the adipocyte growth that occurs in diet-induced obesity [18]. The interpretation of these results are complicated, however, by studies in which AT1R are deleted from aP2-containing cells using the Cre/lox system [80]. This genetic manipulation leads to AT1R reduction within white and brown adipose tissue and heart and also to a metabolic profile in low-fat diet feeding conditions that somewhat contrasts that of the whole body AT1aR knock-out mouse (i.e., there is an apparent reduction in the differentiation of adipocytes coupled with adipocyte hypertrophy). There was no impact of the gene deletion on diet-induced adiposity, however [80]. Although these data do not support a role for adipocyte AT1aR in diet-induced WAT expansion, they do not necessarily rule out such a role. It is possible that the AT1aR deletion within brown adipose tissue or heart overshadow such effects of the gene deletion within white adipocytes. Conversely, some *in vitro* studies again point to a role for Ang-II in facilitating energy storage within adipocytes. For example, upon examining the effects of Ang-II on human adipocytes, Goossens *et al.* determined that activation of the AT1R inhibits lipolysis, an effect that was blocked by Losartan, an angiotensin receptor blocker (ARB) [81]. Furthermore, drugs that inhibit Ang-II formation or action, such as ACE inhibitors and ARBs, have been associated with improved lipid profiles and insulin sensitivity in humans [82–84] and with reduced adiposity in rodents [10].

Decreased AGT levels, and inhibition of Ang-II formation also lead to decreased activation of the AT2R and there is some evidence for a role for this receptor subtype in WAT physiology. Deletion of the AT2R in mice leads to decreased lipogenesis, but increased adipocyte differentiation. The end results are an increase in adipocyte number, a reduction in adipocyte size and consequent improvements in insulin sensitivity and resistance to diet induced obesity relative to wild-type controls [19]. In confirmation of these findings, deletion of the AT2R in mesenchymal stem cells used to study adipogenic differentiation results in increased adipogenesis and a consequent elevation in lipid uptake [85]. Furthermore, when investigating the metabolic role of AT2R activation in adipose tissue, Littlejohn *et al.* observed a decreased thermogenic profile in adipose tissue of mice treated with an AT2R activator, CGP-42112a [25]. Together, these data suggest adipose tissue AT2R activation plays a role in storing excess lipids and suppressing resting metabolism. It is important to recognize, however, that until recently, the literature primarily focused on male models, demonstrating that AT2R contributes to an obesogenic effect. In striking contrast, AT2R deletion in female mice significantly enhances [86], while chronic pharmacological activation of the AT2R, via Compound 21, attenuates indices of energy excess independent of estrogen availability [26]. The implication is that the AT2R may play a key role in the gender differences in metabolism, perhaps acting to dampen or exacerbate energy excess in females or males, respectively.

3.2. ACE2/Ang-(1-7)/MasR Axis in White Adipose Tissue

In contrast to the classic Ang II/AT1R activation leading to increased adiposity by acting at the level of the adipocyte, ACE2/Ang-(1-7)/MasR axis activation has the opposite effect. When examining the MasR knock out mouse, Santos *et al.* found that, while this mouse does not demonstrate an increase in body mass compared to the wild-type controls, they do

display a significant increase in body fat percentage [22]. Furthermore, increased insulin resistance and decreased glucose transporter-4 (GLUT4) expression in adipose tissue, accompany this elevated body fat [22]. We have observed that chronic treatment with Ang-(1-7) in diet-induced obese C57BL6/J mice reduces body mass (Figure 2). Others have studied these effects further and observed that the reduction in body mass is associated with decreased adiposity, and improved lipid profiles and insulin sensitivity [87]. These studies indicate that upregulation of Ang-(1-7) in adipose tissue may be key in the treatment of obesity and metabolic disorders.

Additional studies investigating the ACE2 component of this 'protective arm' in adipose tissue have revealed that the expression and activity of this enzyme are regulated by energy status [23, 88, 89]. For example, Gupte *et al.* determined that ACE2 mRNA expression is increased in adipose tissue of male mice maintained on high-fat diet for 1 week and for 4 months [23, 89]. Moreover, adipose tissue ACE2 activity is elevated after 1 week, and is not maintained for the full 4 months [23, 89]. However, just as with the AT2R, ACE2 may be another component of the RAS, which plays a role in gender differences as they relate to obesity. Unlike their male counterparts, in female mice the increase in ACE2 activity and thus Ang-(1-7) production is maintained throughout the duration of high fat feeding [89]. Interestingly, female mice exhibited a greater body mass and adiposity, compared to their male counterparts [89]. These data suggest, that while chronic administration of Ang-(1-7) in male mice leads to reduction in bodyweight, endogenous increases in ACE2/Ang-(1-7) activity in females may not produce the same effects [89].

Much like manipulating traditional components of the RAS within adipose tissue impacts systems other than adipose tissue, upregulation of the ACE2/Ang-(1-7)/MasR axis in adipose tissue may also have a broader impact [23, 89]. As indicated in the female animals, the protective effect of the adipose ACE2/Ang-(1-7)/MasR axis is not manifested by a reduction of adiposity, but rather by a decrease in blood pressure, presumably secondary to an impact on cardiovascular or neural tissue [23, 89]. This is due to the fact that the local RAS of the WAT is not a closed system. Increased expression of AGT in WAT, leads to increased plasma AGT levels. Conversely, increased adipose ACE2 activity in female mice, results in decreased plasma Ang-II levels and increased Ang-(1-7) levels. As a consequence, alterations in the status of the adipose RAS will impact other systems, such as the cardiovascular and nervous system. In the following sections, we discuss the possibility that RAS also impacts the neural regulation of energy balance.

4. The Renin Angiotensin System in the Neural Regulation of Energy Balance

Although there are tremendous variations in the amounts of energy that individuals consume and/or expend, most animals (humans included) are capable of precisely matching energy intake with energy expenditure such that a particular level of adiposity is defended. The brain plays a critical role in maintaining this balance by receiving, integrating and responding to peripheral signals regarding energy status [30, 31]. Despite the tremendous accuracy of this homeostatic system, certain disruptions, such as the exposure to stress or the

chronic ingestion of calorically-dense foods, often shift the balance of the system to defend either a higher or lower level of adiposity. Along these lines, Professor Sakai contributed substantially to understanding the impact of social stress on metabolism [90–94]. Furthermore, numerous endocrine factors are known to influence this neural regulation and the RAS has similarly been implicated in these processes [20, 21, 28, 29, 95].

As discussed above, metabolic disturbances, such as obesity, often lead to increased levels of circulating Ang-II that arise, at least in part, from adipose tissue AGT [51, 74], while weight loss has the opposite effect [4, 71, 96]. In addition to the peripheral actions of the RAS, these increases in Ang-II are also speculated to act in a negative feedback manner on the brain Ang-II receptors in attempt to offset the increased adiposity encountered during obesity [5, 9, 51, 74]. Along these lines, it is important to note that although, in general, mice with genetically deleted components of the traditional RAS are lean, they are also, in some cases, hyperphagic. This phenotype is consistent with a peripheral role of the RAS in promoting energy storage, as described previously, and also a potential central role of the RAS in promoting negative energy balance. In further support of this notion, increasing the levels of Ang-II specifically within the CNS, either through genetic or pharmacological approaches leads to reduced body weight and adiposity [10, 20, 21, 28, 95, 97, 98], while genetic inactivation of Ang-II actions in the brain positively regulates certain aspects of energy balance [99, 100].

In regards to the impact of the RAS on energy consumption, peripheral or central infusion of Ang-II leads to a reduction in caloric intake that is associated with altered hypothalamic expression of genes known to regulate energy balance (e.g., corticotrophin-releasing hormone [CRH] and thyrotropin-releasing hormone [TRH]) [21, 29]. Conversely, transgenically reducing brain RAS activity via the brain expression of antisense oligonucleotides targeting AGT, elevates food intake [99]. While these previous studies clearly revealed an impact of Ang-II on food intake they did not explicitly determine whether or not the observed decreases in food intake were due to direct actions on the neural circuitry involved in food intake regulation or if they were secondary to alternative actions of Ang-II (e.g., its impact on hydromineral balance or stress-related behavior). Furthermore, there is also evidence indicating that food intake is not altered by manipulation of the RAS [20, 101]. Therefore, the mechanism(s) and extent by which Ang-II is involved in food intake regulation is far from clear.

Only slightly more clear is the notion that elevated Ang-II levels within the brain increase energy expenditure, as well as indices of sympathetic activation of brown and white adipose tissue, which may indicate increased thermogenesis and lipolysis [10, 20, 21, 28, 95, 97, 98]. Although this holds true for both the systemic and the central infusion of Ang-II [28, 102–104], this contention is still complicated by data collected from genetic mouse models that lack components of the RAS [12, 17, 18]. By and large, whole-body genetic deletion of various RAS components leads to negative energy balance that is associated with elevations in energy expenditure [17, 18] or increased locomotor activity [12]. These models of course are not specific to the central RAS, and it is possible that the impact of peripheral deletion or downregulation of the RAS overshadows any possible effect of central RAS. Along these lines, and in support also of a stimulatory role for Ang-II in energy expenditure, rats

subjected to a brain-specific deficiency in AGT exhibit a reduction in energy expenditure [105]. Furthermore, Grobe and colleagues have conducted a series of studies revealing that the enhanced central RAS actions at the AT1R are required for the elevated energy expenditure and consequent negative energy balance that is associated with the deoxycorticosterone acetate (DOCA)/ salt model of 'neurogenic' hypertension [106].

Based on these collective observations it is apparent that there exists a divergence between the brain- and adipose-specific effects of the RAS on energy balance. This disparity is suggestive of the presence of a negative feedback system that is activated when adipose AGT levels are high, allowing Ang-II from adipose tissue to enter circulation and gain access to the CNS, providing a brake on peripheral Ang-II action. This is strikingly similar to the actions of insulin and leptin, which are both widely accepted adiposity signals. The circulating levels of leptin and insulin become elevated in obesity and act within the arcuate nucleus of the hypothalamus in a negative feedback manner to reduce energy storage. Although the end-point is essentially same (i.e., insulin, leptin and Ang-II all act centrally to promote negative energy balance), it is possible that the neural circuits and molecular mechanisms that underlie Ang-II-induced negative energy balance are distinct from those that mediate insulin and leptin actions, and the current literature assessing this possibility is discussed in the subsequent section.

4.1. Angiotensin-II's Actions at Brain AT1R and AT2R

Both the type-1 and type-2 angiotensin receptors are positioned within brain nuclei integral to the regulation of energy consumption and of energy expenditure [107, 108]. For example, AT1R are densely-localized to and/or influence the activity of both neurosecretory neurons and preautonomic neurons of the parvocellular PVN, some of which are known to express CRH and TRH [109–114]. Elevation of brain Ang-II levels increases CRH and TRH expression [21, 112], and there is evidence indicating that these factors promote weight loss via inducing anorexia and/or elevating energy expenditure [29, 115–117]. Conversely, deletion of PVN AT1aR, leads to a reduced propensity to develop high-fat diet-induced blood pressure elevations, while also leading to increased food intake and reduced energy expenditure, ultimately culminating also in an enhanced susceptibility to diet-induced obesity [100].

Another line of evidence that supports the divergent actions of the brain and adipose RAS, as well as the involvement of PVN AT1aR in the regulation of energy balance, comes from a set of studies that were conducted in collaboration with Randall and focused on the impact of the systemic administration of captopril on energy metabolism [10]. Captopril is an ACE inhibitor that prevents the formation of Ang-II in circulation but does not readily access the brain. In these studies, we determined that rats given captopril weighed less and had less body fat than controls and comparisons to pair-fed controls indicated that this attenuated weight gain was primarily a consequence of reduced food intake [10]. Using this model, peripheral ACE activity was suppressed; however, because captopril elevates plasma and consequently brain Ang-I (the precursor for Ang-II and substrate for ACE), but does not itself enter the brain [118–120], we hypothesized that still-active brain ACE would convert increased Ang-I into Ang-II, and that increased central Ang-II would contribute to systemic

captopril-induced negative energy balance. Consistent with this notion, the reduction in food intake elicited by peripheral captopril was reversed by co-administration of the ACE inhibitor into the brain [10]. Not only did Randall play an important role in the studies included in the manuscript itself [10], but he also suggested that we follow-up on these studies by examining the impact of PVN AT1aR deletion using the approach in [100] on the negative energy balance induced by captopril - the hypothesis being that the PVN AT1aR are necessary for captopril-induced weight loss. Consistent with this hypothesis, captopril-induced body and adipose mass loss is attenuated in mice that lack the PVN AT1aR relative to their control counterparts expressing only the AT1aR flox gene (Figure 3). The point is that, with Randall's help, we discovered that AT1R within the PVN are likely a fundamental component of the brain RAS circuitry that controls metabolic function. That being said, it is unlikely that the AT1aR in the PVN represent the only mechanism by which Ang-II can impact metabolism.

Other brain regions that are involved in body weight regulation and also contain AT1R are the arcuate nucleus of the hypothalamus (ARC; see Figure 4), the dorsal medial hypothalamus (DMH) and the nucleus of the solitary tract (NTS), among others [107]; and there is some evidence that AT1R may impact energy balance via actions at these receptors. Furthermore, it is also apparent that there exists a certain level of cross-talk between Ang-II, and the traditional adiposity signals, insulin and leptin, which are known to act at these brain regions to impact energy balance [7, 15, 101, 121–126]. For example, there are some studies indicating that Ang-II modulates the expression of factors within the ARC (e.g., pro-opiomelanocortin and agouti-related peptide) [21, 127] that are responsible for leptin and insulin-induced anorexia and elevated energy expenditure. Antagonism of the AT1R using telmisartan enhances leptin sensitivity in lean and obese rats [122] and Xue *et al.* elucidated another leptin/ Ang-II interaction by determining that high-fat diet-induced sensitization of Ang-II induced hypertension is mediated by leptin's impact of the central RAS system and proinflammatory cytokines [7]. Furthermore, Young *et al.* determined that Ang-II and leptin interact at the level of the subfornical organ to influence brown adipose tissue thermogenesis [101]. In regards to insulin, there is substantial overlap between its and Ang-II's signaling cascades [125, 126] and, in the periphery, Ang-II decreases insulin sensitivity [15, 123–125]. Similarly, in the brain, the blockade of AT1R signaling attenuates the pressor response to insulin [128]. The inference is that Ang-II signaling in the brain is required for insulin-induced blood pressure elevations.

Another intricacy of Ang-II's potential involvement in the neural regulation of energy balance is that this peptide may also influence these processes via actions at the AT2R. Similar to their AT1R counterpart, AT2R are localized to brain regions that are known to influence metabolism and there is evidence for beneficial actions of AT2R on metabolic function [108]. For example, one such brain region to which AT2R are densely-localized is the NTS which, in addition to its influence on cardiovascular function, has a clear role in the regulation of metabolism [129, 130]. Furthermore, the dorsal motor nucleus of the vagus (DMNV) is widely recognized to impact metabolic function via vagal input to the pancreas and other organs comprising the gastrointestinal tract [131]. AT2R is localized to the DMNV and Ang-II has previously been found to excite neurons within this region [132]. It is also of relevance to note here that although AT2R are not densely-localized to the energy balance

regulating ARC and PVN, there are particular abundances of AT2R-eGFP containing terminals and/or fibers within these regions (see Figure 4 and [108, 133]). While within the ARC the practical relevance of these AT2R-eGFP terminals is not yet known, we have previously observed that these AT2R exert an inhibitory (GABA-mediated) influence over vasopressin neurons within the PVN [133]. It is possible that AT2R localized to nerve terminals within the ARC similarly impact the activity of neurons.

Functionally and in regards to energy balance, rats that overexpress AT2R have a lower body weight [134] and AT2R activation using the selective agonist, Compound 21, reduces body weight [26], improves glucose tolerance [135] and impacts insulin biosynthesis and secretion [136]. Additionally, Ohinata *et al.* utilized another pharmacological approach (i.e., the AT2R antagonist, PD123319) as well as AT2R knock-out mice in order to reveal a potential role of brain AT2R the reduction in food intake induced by angiotensin peptides [137].

Collectively, these studies indicate that that AT1R and AT2R within the CNS likely contribute to neural control of metabolism. Still, the neuroanatomical mechanism(s) behind these actions are far from clear.

4.2 ACE2/Ang(1-7)/MasR axis

The ACE2/Ang-(1-7)/MasR axis influences the neural regulation of energy metabolism via at least two general mechanisms. First, alterations in the levels or activity of ACE2 may impact energy metabolism via influences on the levels of Ang-II and, consequently, the AT1R and AT2R actions discussed above. Second, ACE2 also elevates Ang-(1-7) levels which can then activate the MasR in various brain regions. This Ang-(1-7)/MasR pathway has been implicated in various brain functions, including anxiety-like behavior, the neural regulation of cardiovascular function and neuroendocrine secretion, among many others [138, 139]. Consistent with a role for this protective axis in the neural regulation of energy balance, ACE2 has been localized to many relevant brain regions such as the PVN, arcuate and NTS [140]. Similarly, immunohistochemical and *in situ* hybridization studies have localized the MasR to many such brain regions [141–143] and we have detected MasR mRNA in the basolateral amygdala [138].

In regards to the neural regulation of energy balance, there is substantial evidence that Ang-(1-7) activation of the MasR promotes insulin sensitivity [95, 144, 145] and insulin signaling in the brain is known to decrease appetite [146–149]. As a consequence, it is reasonable to hypothesize that Ang-(1-7) may potentiate insulin's anorectic actions. That being said, it is also important to realize that there are numerous published studies in which this 'protective' axis is manipulated genetically (or pharmacologically) and throughout entire body and no alterations in body mass or food intake are observed [22, 87, 150]. Therefore, it will be essential to determine the brain-specific actions of the Ang-(1-7)/MasR in the regulation of energy balance so as to determine whether or not this axis shows promise as a novel therapeutic avenue for obesity intervention.

5. Conclusions

As the complexity of the RAS continues to be uncovered so do the intricacies of its involvement in the regulation of energy balance. The RAS receptors within adipose tissue and brain are positioned such that the effector peptides of the RAS may directly impact metabolism; however, their roles in these processes are far from clear. In this manuscript, we discussed previously-published studies and new data that support a role for the RAS in metabolic function, one of Randall's many scientific interests. The overarching theme is that the RAS can have diverse actions on metabolic function, depending not only of the component of the RAS that is manipulated, but also depending on the specific tissue in which the RAS is stimulated.

While the focus here is on the metabolic effects of the RAS, it is also important to note that Randall made many tangible contributions to understanding the role of the RAS in stress responses [151, 152] and salt appetite [153–163]. That being said, the contribution that is, perhaps the most valued by those who had the privilege of working with him, is his devotion to his trainees. Randall was a key member of my (Annette's) dissertation committee and he certainly influenced my development as a scientist during graduate school. For example, I will never conduct an experiment without first questioning whether or not Randall would approve of the quality (and quantity) of the controls that I intend on including. Although he was and continues to be important to me from a scientific standpoint, he was much more than a mentor and colleague to me. He was family; and my impression is that he had a similar role in the lives of many other scientists. At meetings, he was always the first to introduce me to his colleagues and took care to include me in as many professional networking events as possible. On a more personal level, Randall always made me feel welcome. He did not hesitate to bring my husband and I into his home on numerous occasions both in times of need and for great parties. He was there for me professionally and personally. Thank you Randall; you are and you will continue to be sorely missed.

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Figure 1. The Updated Renin-Angiotensin System (RAS)

Angiotensinogen (AGT) is converted to Angiotensin I (Ang I) by Renin. Ang I is further converted to Ang II, by ACE, and most commonly acts upon the AT₁R. Ang II also acts at the AT₂R and often counterregulates AngII/AT₁R actions. The often referred, “protective arm” of the RAS converts Ang II to Ang-(1-7) via ACE2. Ang-(1-7) stimulates the MasR and, much like the AT₂R, acts in opposition of the AT₁R.

Figure 2. Subcutaneous administration of Ang-(1-7) reduces diet-induced body mass gain
Chronic administration of 0.3 ug/h of Ang-(1-7) in diet-induced obese mice led to a significant reduction in body mass. Analysis is 1-way ANOVA. * = $p < 0.05$, significantly different from control.

Figure 3. Deletion of the AT1aR within the PVN dampens the reduction in body mass and adipose mass gain induced by the ACE inhibitor, captopril PVN AT1R KO mice (described in [100]) and littermate controls expressing only the AT1R flox gene (CON) were given captopril (CAP) in their drinking water or were given standard drinking water (VEH) as described in [10]. Body mass and adiposity were then evaluated as in [100]. (A) The change in body mass during the 20 day study (a = CON-CAP significantly different than CON-VEH, $p < 0.05$; b = CON-CAP significantly different than PVN AT1R KO-CAP, $p < 0.05$; c = CON-CAP significantly different than PVN AT1R KO-VEH; d = PVN AT1R KO-VEH significantly different than KO-CAP. Significance is considered $p < 0.05$, two-way repeated-measures ANOVA, Bonferroni post-tests). (B) The change in adipose mass at the end of the 20 day study (* = $p < 0.05$).

Figure 4. Expression of AT2R-eGFP and AT1aR-tdTomato within the arcuate (ARC) and adjacent ventromedial nuclei (VMH) of the hypothalamus
Projection images of the ARC and VMH of a dual AT2R-eGFP and AT1aR-tdTomato reporter mouse depicting (a) AT2R-eGFP in green, (b) AT1aR-tdTomato in magenta and (c) the merged image. 3v = third cerebral ventricle. Scale bars = 100 μ m.