Chapter

1

Neuronal Control of Aging
Hormonal Signaling and Metabolic Pathways

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Metabolic Rate and Genomic Stability

Nearly 100 years ago, in 1908, Rubner postulated an inverse relation between metabolic rate and lifespan on the basis of a comparison among mammalian species (e.g. 16 KJ/day in mice vs. 7200 KJ/day in humans). These observations laid the foundation of Pearl's "rate of living" theory of aging[1]. Indeed the rate of living or the metabolic rate has been linked to the production of partially reduced oxygen species, the byproducts of oxygen metabolism. Approximately 2-3% of oxygen used by cells is chemically reduced by addition of single electrons which sequentially generate superoxide anion ($.O_2$-) and H_2O_2, leading to DNA damage which needs to be repaired efficiently and effectively. The scission of H_2O_2 leads to the formulation of hydroxyl free radicle (.OH), one of the most reactive molecules. The damage to DNA by free radicals, and the rate of repair of damaged DNA, determine the genomic stability. An animal's ability to repair certain types of DNA damage is directly related to the lifespan of that species. For example, the humans repair DNA more quickly and with higher efficiency than the rat or other animals with shorter lifespans[2].

Endocrinal, nutritional and environmental factors may result in an increase in production of free radicals or a decrease in the efficiency of DNA repair mechanism. The net effect is an alteration in the genomic stability which would accelerate the rate of aging. Another significant observation that needs highlighting is the fact that the time to reproductive maturity (ability to procreate) from fertilization is directly related to longevity[3]. The longer the maturation time, the longer is the lifespan. This holds good for nearly all species, with only a few exceptions.

It, therefore seems appropriate that feeding behavior, as an important determinant of metabolism and sex behavior as a determinant of procreation, constitute major determinants of longevity. It is obvious that hormones and metabolic pathways that are involved in ***both*** these processes, namely feeding behavior and sexual behavior (reproduction) are likely to provide the base and basis of metabolic connectivity between nutrition intake and aging. Most appropriately the regulatory and control mechanism for both these processes are located in the hypothalamus.

Neuronal Regulation of Feeding Behavior

Insulin and its signaling systems play a major role in both central and peripheral mechanisms which are involved in the control and regulatory mechanisms governing intake, metabolism, and storage of nutrients not only in the human but in a large number of living organisms.

Upto the late 60s and early 70s, the standard textbooks of physiology stated that insulin does not affect glucose metabolism of the brain, and that insulin does not cross blood-brain barrier. However, our collaborative work during that period challenged this concept[4]. We showed that in male cats evoked responses from ventromedial hypothalamus (VMH) and lateral hypothalamic area (LHA), following stimulation of mesenteric nerves, were modified by IV injection of glucose or insulin. Glucose produced initial decrease in amplitude, which gradually recovered while insulin produced an initial short acting inhibition followed by an increase in amplitude. These studies emphasized the relationship between net rate of neuronal glucose utilization as an important determinent of the amplitude

of evoked responses from VMH and LH[5]. These observations also reinforced the data from the same laboratory published earlier[6]. Finally, stimulation of LH increased food intake while stimulation of VMH produced opposite effect.

We hypothesized that as insulin influenced the activity of VMH and LH neurons, these neurons may likewise affect the insulin release from pancreas. The studies were designed to investigate this. It was shown that in conscious restrained rhesus monkeys, stimulation of LHA increased serum insulin while stimulation of VMH decreased the levels of circulating insulin. Based on this and additional experimental data, we proposed in 1976 the existence of entero-hypothalamo-insular axis[7]. The last three decades generated an intense scientific debate, culminating into a conceptual model which is now generally accepted. The subject has been extensively reviewed[8].

Insulin and Hypothalamus: Current Concepts

There is increasing evidence that in the brain, insulin is involved in a wide array of regulatory mechanisms including neuronal survival, neuronal plasticity, learning and memory, as well as energy homeostasis and reproductive endocrinology.

Essentially, glucosensing neurons are predominantly located in those areas of brain that are involved in the control of neuroendocrine function, nutrient metabolism, and energy homeostasis. A select group of such neurons use glucose as a signaling molecule to alter their firing rate both as a means of, and also as a response to, glucosensing. The VMH contains both the ventromedial hypothalamic nucleus (VMN) and the arcuate nucleus (AN). Both contain glucosensing neurons that respond to changes in ambient glucose levels. Through effector pathways, these neurons are involved in regulation of glucose homeostasis. The population of glucosensing neurons in the VMH (VMN and AN) is amongst the best characterized with respect of glucosensing. In the VMN, 14-19% neurons are glucose-excited (GE) and 3-14% are glucose-inhibited (GI) in type[9]. Glucosensing neurons use glucose in a concentration dependent manner as a signaling molecule to regulate their membrane potential and action potential frequency[10]. It has been suggested that GE neurons are analogs of pancreatic β-cell, whereas GI neurons have some similarities to α-cells: GE neurons and β-cells are activated and GI neurons and α-cells are inhibited by increase in ambient glucose levels[11]. The LH contains predominantly glucose-inhibited neurons[12].

Insulin Signaling in Hypothalamic Neurons

Glucokinase (hexokinase IV) is a key regulator of neuronal glucosensing, thus performing a role similar to that in the pancreatic β-cell (and α-cell) glucosensing[13]. In the arcuate nucleus, more than 75% of neuropeptide Y-(NPY-) positive neurons express glucokinase[14]. Many glucokinase-expressing neurons coexpress K_{ATP} channels[15]. Furthermore, coexpression of GLUT-3 and GLUT-4 with insulin receptor mRNA (IR mRNA) is also reported in glucose-responsive neurons[16]. Recent studies confirm that glucose-excited neurons utilize ATP-sensitive K^+channels as their transduction mechanisms for glucosensing whereas glucose-inhibited neurons appear to utilize a nonspecific Cl^- channel. Irrespective of the type of ion channels used as a final common pathway, a large proportion of glucose-excited and inhibited neurons appear to utilize glucokinase as a regulator of glucosensing. Glucokinase mRNA is selectively localized in several brain areas involved in glucosensing. It is expressed ~ 70% of GE and ~ 40% of GI neurons[16]. Final confirmation of the key role of neuronal glucokinase is based on the fact that a knock down of glucokinase mRNA, using glucokinase siRNA in primary hypothalamic neuronal cultures, ablates the ability of these neurons to sense glucose[17].

Insulin and Reproductive Function

The role of insulin in reproductive physiology has been shown by the observation that an increase in the circulating levels of insulin by hyperinsulinemic clamp study in male mice, resulted in a significant rise in gonadotropin-releasing hormone (GnRH) secretion. This effect of insulin was likely mediated at the hypothalamic level as a similar stimulation of secretion and expression of GnRH by insulin was also demonstrated in hypothalamic neurons in culture[18]. It is suggested that such an action of insulin constitutes an essential mechanism for the adaptation of reproductive function to changes in the nutritional and metabolic status of an organism / individual.

Insulin Signaling in CNS:Evolutionary Perspective

Insulin signaling in neuronal cells plays a key role not only in mammals but also in primitive organisms such as the nematode *Caenorhabditis elegans and the fruit fly Drosophila melanogaster*[19]. Indeed, insulin signaling pathways shows several similarities in *C. elegans, D. melanogastar*, rodents, and humans, thus underscoring the possibility of an evolutionary mechanism conserved over the millennia. Neurosecretary cells in *D. melanogaster* express insulin-like peptides (dILPs). Ablation of

dILP neurons results in prolonged lifespan, reduced fertility, increased fasting glucose levels, increased storage of lipids and carbohydrates, and reduced tolerance to heat and cold, thus highlighting the key role of these cells in the regulation of lifespan and fuel metabolism[20].

Additional discoveries in this connection have clarified the role of insulin signaling in providing metabolic connectivity between nutrition, reproduction and lifespan.

The initial discovery in *C. elegans* was the cloning of *DAF-2,* the gene that encodes a homologue of the mammalian insulin receptor, containing both ligand binding and tyrosine kinase domains[21]. The relevance of DAF-2 to *C. elegans* physiology was initially based on its association with a stage of diapause arrest called "dauer." Entry into this developmental stage, characterized by inhibition of reproduction and reduced metabolism and growth that resembles suspended animation or hibernation, is normally triggered by periods of reduced food availability. Mutations of DAF-2 were shown to produce the dauer state and also revealed DAF-2 as the first step in a signal transduction cascade homologous to the insulin pathway described in mammals. One of the proteins in signal transduction pathway is called advanced glycation end product (AGE)-1, a homologue of mammalian phosphatidyl-inositol 3-kinase (PI 3K). The knock-out of PI 3K induces the same dauer stage phenotype characterized by increased longevity as seen with mutation of DAF-2[21]. Another key protein is DAF-16, a member of the forkhead transcription factor family related to mammalian HNF-3 and FOXO1. The finding that DAF-16 mutation completely reversed the phenotype arising from DAF-2 or AGE-1 knock-out[22] (a phenomenon referred to as genetic complementation) suggests that this forkhead protein functions downstream of the more proximal DAF-2 and AGE-1 proteins, and that its activity is normally inhibited by activation of the upstream DAF-2/AGE-1 cascade, and finally that this inhibition is a dominant component of signaling in this cascade.

It was suggested that increased longevity associated with the DAF-2 knock-out is analogous to the effect of caloric restriction to increase mammalian longevity, since calorically restricted animals experience decreases of both circulating insulin (and hence reduced insulin receptor signal transduction) and fertility. Restoration of DAF-2 in neurons was sufficient to restore lifespan and reproduction of DAF-2 knock-outs to wild-type values, and neuron-specific restoration of AGE-1 in animals that otherwise lack this protein produced the same effect. Thus, neuronal insulin-like signaling appears to be a key regulator of various critical functions in *C. elegans*, and the metabolic and reproductive defects induced by whole-body deletion of DAF-2 appear to be attributable in large part to abnormalities specific to the absence of insulin-like signaling in the nervous system.

In 2001, an analogous set of studies investigated the insulin receptor-like signaling system in the fruit fly, *Drosophila*. It is of interest to note that as of March 2002[23], *Drosophila* gene sequences have been found with highly significant ($P < 10^{-10}$) matches to 75% of the human disease loci examined. It is amazing, therefore, that fully 75% of human disease loci have counterparts in *Drosophila*.

While the insulin-like receptor was first reported in this species in 1996, knock-outs were found to be lethal; hence, insulin receptor-like activity is absolutely essential for life during development. However, mutations of either an Insulin Receptor Substrate (IRS) homologue termed CHICO, or complex heterozygotes of the insulin-like receptor, were shown to extend lifespan and reduce reproduction in a manner similar to that induced by DAF-2 mutants in *C. elegans*[24]. As in *C. elegans*, lifespan extension was associated with a general growth deficiency and a decrease in cell number and size, and insulin-like signaling was shown to depend on a PI3K homologue. It is suggested that an early evolutionary role for insulin may have been to regulate metabolism through neuronal control of nutrient storage, a process tightly coupled with control of reproduction and lifespan, since both energy storage and reproduction depend upon nutrient availability. According to this hypothesis, the emergence of insulin as a key regulator of carbohydrate metabolism in vertebrates was a more recent evolutionary development.

Extending the evolutionary perspective, the intimate relationship between immune and metabolic responses also needs to be highlighted. It is well recognized that functional structures that control key metabolic and immune functions have evolved from common ancestors. An oft-quoted example is *Drosophila* fat body, which contains the mammalian homologs of liver, the hemapoietic system, and other immune components. It has been recently shown that this site also corresponds to mammalian adipose tissue[25]. As these specialized cells differentiate into distinct functional units or organs, they carry with them their developmental heritage. Hence, it is possible to envision a scenario where common pathways regulate both metabolic, reproductive and immune functions through the utilization of common

key regulatory molecules such as glucose and fatty acids. It is of interest to note that glucosensing neurons in VMN, AN and LHA also respond to a variety of metabolites such as lactate, ketone bodies and free fatty acids[26,27]. Long chain acyl-CoA also activates K^{ATP} channel in these neurons[28] and inhibits GK activity[29]. Thus glucosensing neurons indeed function as ***metabolic sensors***. Such a closely linked configuration and coordinated regulation of metabolic and immune responses is likely to be advantageous, since the organism needs to organize and redistribute its metabolic resources during the mounting of an immune or inflammatory response.

In summary, in response to the taste and/or ingestion of food, one or more insulin ligands are released from neurosecretory cells in the CNS. Activation of the insulin receptor homologue, DAF-2, in CNS and elsewhere triggers intracellular signaling via the PI3K homologue AGE-1. The ultimate consequence of these events is the inactivation (by phosphorylation) of the forkhead transcription factor, DAF-16, an effect that permits normal growth, aging, reproduction, and fat storage. This cascade of events can be interrupted by inadequate nutrient availability, leading to reduced insulin signaling, activation of DAF-16, and subsequent entry into the dauer phase of development. Such animals are characterized by reduced growth and reproductive capacity and increased longevity and fat mass. Inactivating mutations of DAF-2 or AGE-1 also induce the dauer condition, and these effects are blocked by inactivation of DAF-16.

Insulin Signaling and Aging

Insulin signaling in the CNS of mammals, including humans, seems to have many biochemical, molecular, and physiological parallels with its role in invertebrates. For example, caloric restriction in mammals is associated with reduced secretion of insulin and reproductive hormones (e.g., follicle-stimulating hormone and luteinizing hormone) and, if prolonged, with extension of lifespan[30]. Energy-restricted states are also associated with decreased adipose tissue mass and, consequently, of plasma leptin and insulin concentrations as well. Since insulin is a major regulator of leptin biosynthesis and release from adipocytes, low plasma concentrations of both hormones are usually found together and are associated with similar outcomes. Therefore, low levels of insulin may be the critical factor in prolonging life in mammals as well as invertebrates[31,32].

The downstream pathways controlling longevity and reproduction may be independent of one another[33]. This conclusion is based on the suppression of DAF-2 activity in adult *C. elegans* (after the development of reproductive capacity) via application of RNA*i*. In these animals, lifespan is extended despite suppression of insulin-like signaling that was intact until a late stage of development. Furthermore, reproductive timing was specified independently of the dauer decision, suggesting that the DAF-2 pathway can function late in development to affect the timing of reproduction[33]. Thus, while extension of longevity appeared at first to be invariably associated with impaired growth or reproduction, selective manipulation of this pathway may permit youthfulness and lifespan extension without affecting these other processes.

Caloric Restriction and Longevity

Understanding of basic mechanisms underlying hormonal pathways involved in effecting aging proess by means of caloric restriction have resulted in a major resurgence of interest in biology of aging during last 5-7 years. Caloric restriction (CR) is now recognized as a nutritional intervention with beneficial effects on longevity. Both in rodents as well as in primates, CR produces physiological changes resulting in reduced body size, lower body temperatures, decrease in levels of blood glucose alongwith a decrease in insulin, growth hormone, and IGF-1 levels. In addition, there are effects on reproductive development including delay in sexual maturation and decrease in fertility. Of critical importance is the modification of the insulin/IGF-1 signaling pathway in CR annals[34]. It is interesting to note that insulin supplementation can reverse the effects of CR on mitochondrial free radical production in liver[35] as well as on lipid peroxidation in liver and heart[36].

Epilogue

In 1993, while writing the Foreword for Current Concepts in Internal Medicine, I had observed:

*'I visualize a resurgence of interest in providing an integrated theory **linking neuroendocrinology, nutrition and metabolism**. There is enough evidence to suggest that dietary restriction may be an important antidote to aging, provided this approach is followed throughout life, and certainly from early adulthood. It has also been shown that older subjects who regularly do aerobic exercise perform better in cognitive tests than age-matched sedentary individuals. Could this be the key to the longevity and the quality of life of our ancient Rishis, who practiced dietary restriction and Yoga throughout their life!*

The crucial question that we may have to carry to the next millennium may not be regarding the nature of changes in the aging brain, but the reason as to why these changes are so selectively heterogeneous. For example, why is it that in the

aging brain very few neurons disappear from those areas of hypothalamus that synthesis and release hormones which are trophic to the pituitary, while a large number of neurons disappear from the substantia nigra and locus coeruleus? Lastly, why is the neuronal loss in the hippocampus so regulated that it approximates about 5% of the total neurons in this area during each decade in the second half of life? As it is, the limbic system is essential to learning, memory and emotion. In preprogramming the quantum of neural loss at a low rate, is nature being merciful to man'?[37]

The forecast reflected in the first paragraph cited above is now more than amply supported by the data cumulated over the last decade and presented in the text. The questions posed in the second paragraph still await resolution.

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