Soybean Amino Acids Excite Orexin Neurons

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Abstract

Soybeans contain a plethora of peptides, nutrients, and phytochemicals that are beneficial to human health. They have been studied as a possible treatment and prevention against many diseases including cancer, obesity, and cardiovascular disease. The hypothalamus requires many of the amino acids found in soybean to maintain proper functioning of neurocircuit systems, in particular the orexin system. The orexin system is a crucial component to the human brain. Orexin neurons are known to modulate the sleep-wake cycle, feeding, emotions, autonomic nerve activity, and homeostasis. When orexin is deficient, disorders such as narcolepsy occur. Many humoral factors influence orexin neuron activity, one of which is amino acids. This review will focus on the potential excitation of hypothalamic orexin neurons by soybean amino acids.

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Introduction

Soybeans (*Glycine max*) have been a staple source of protein for thousands of years. Recently, there has been a growing interest concerning the association of biologically active peptides in soybeans with positive health benefits. Research has shown that soybean bioactive peptides have the potential to lower the risk of chronic diseases such as cancer and cardiovascular disease. Soybean protein provides all nine essential amino acids required for the proper functioning of the body's organs and many of its systems, including the brain and brain functions. Of particular focus is the hypothalamus, a region of the brain that maintains homeostasis and produces peptides. This region aids in the regulation of vital body functions such as heart rate, body temperature, appetite and the sleep-wake cycle. Neurocircuit systems located in the hypothalamus, specifically the orexin system, can be inhibited by a deficiency or excited by an excess of amino acids, many of which are found in soybeans.

The orexin system regulates sleep, arousal, energy homeostasis, feeding, and motivation (Tsujino & Sakurai, 2013). Orexin peptides are neurotransmitters produced by orexin neurons located in the lateral hypothalamic area (LHA) (Tsujino & Sakurai, 2009). From this exclusive location, orexin neurons project widely to stimulate most of the brain, with major inputs to arousal and reward centers, where orexin neuropeptides are released and act on two specific G protein-coupled receptors. The firing of orexin neurons promotes wakefulness and is critical for maintaining consciousness (Karnani et al., 2011). The loss of these neurons can cause disorders such as narcolepsy and obesity.

In addition to the functions listed above, orexin cells are also able to “monitor and respond to humoral and neural indicators of energy balance” (Tsujino & Sakurai, 2013). “By exhibiting inhibitory responses to key indicators of energy levels, such as glucose and leptin and excitatory responses to other major macronutrients, such as amino acids or other typical dietary mixtures of macronutrients, orexin neurons form a link between vital functions and body energy status” (Tsujino & Sakurai, 2013). The focus of this review will be on the activating effects of soybean amino acids soybeans on orexin neurons.

Soybean Composition

Nutrition Profile

Soybean is a nutrient rich species of legume native to East Asia. The cultivation of this edible bean has spread to various countries worldwide including Brazil, Argentina, India, Paraguay, Canada, and the United States. In addition to soybean’s high dietary protein, it also contains oligosaccharides, dietary fiber, phytochemicals, and minerals (Table I) (Mateos-Aparicio, Redondo-Cuenca, Villanueva-Suárez, & Zapata-Revilla, 2008). Not only are soybeans considered to be one of the most abundant plant sources of dietary protein, but they are also one of the only plant sources of complete protein (*Soybean nutrition.*2014). Soybean has significant amounts of the essential amino acids, which must be provided to the human body due to the body’s inability to synthesize them. Chart 1 features a comparison of the amino acid content of soybean, corn, wheat, and black bean.

Amino Acids

Soybean peptides are rich in amino acids such as leucine (23.6%), valine (12.22%), lysine (10.01%), isoleucine (8.8%), and phenylalanine (8.75%) (table II). Also found in soybean peptides are aspartate (4.74%) and glutamate (5.55%), both of which aid in performing exercise and delaying fatigue. (Liu & Pan, 2011) indicated “the amount of amino acids, especially alanine, glycine, gamma-aminobutyric acid, isoleucine, threonine, serine, and tyrosine, in the plasma decreases rapidly during endurance testing.” In turn, steady supplies of the aforementioned amino acids are detrimental for proper functioning and maintenance of the human body. Through the orexin system, amino acids can increase energy expenditure and vigilance.

Orexin System

Orexin and Orexin Receptors

Orexins form a unique family containing two neuropeptides (33-amino acid Orexin A and 28-amino acid Orexin B) both of which are produced from a common precursor polypeptide, prepo-orexin. The amino acid sequence and composition of orexin are shown respectively in figure 3 and table IV. Orexin A and B exhibit 11% homology in their N-terminal region, which is known to have little contribution to binding at the receptors (Takai, Tomoyo 2006). The C-terminal region of orexin A contains hydrophobic and hydrophilic residues. The nine residues located on the hydrophobic surface of orexin B are conserved, enabling 68% homology with orexin A for the C-terminal region. (Takai, Tomoyo 2006) suggests that the aforementioned residues “form the surface responsible for the main hydrophobic interaction with the receptors”. One truncation study’s data suggests “Tyr^{17}, Leu^{20}, Asn^{25} and His^{26} play a key role in the interaction of orexin A (17-33) with orexin-1 receptor” (German, Nadezhda A 2013).

The activities of orexin are mediated by two orphan G-protein-coupled receptors (GPCR), orexin-1 receptor (OX1R) and orexin-2 receptor (OX2R) (Tsujino & Sakurai, 2013). OX1R has a higher affinity for orexin A than for orexin B, while OX2R has similar affinity for both orexin A and orexin B. Orexin-producing neurons are located within the lateral hypothalamic area (LHA). Figure 1 presents the distinct areas of the brain that receive signals from lateral hypothalamic orexin neurons (Inutsuka & Yamanaka, 2013).

Orexin-producing Neurons

Orexin neurons are known to play crucial roles in the regulation of sleep-wake cycle and energy homeostasis. (Tsuneki, Wada, & Sasaoka, 2012) establish that “orexin neurons can directly sense the nutritional status by responding to peripheral metabolic signals such as glucose, leptin, and ghrelin” (Figure 1). Research has proven that ghrelin, amino acids, and a decrease of glucose excite orexin neurons, while leptin and an increase of glucose inhibit orexin neurons (Tsujino & Sakurai, 2013). Table III presents a list of factors that influence the firing rate or the membrane potential of orexin neurons; these factors suggest that orexin neurons are “specialized sensors of the internal environment” (Tsujino & Sakurai, 2013). Not only does orexin deficiency throw the equilibrium of the internal environment into disarray, but it also disrupts the circadian cycle.

Orexin Deficiency

Narcolepsy is a sleep disorder characterized by a primary disorganization of behavioral states. This disorder affects approximately 1 in 2000 individuals in the United States (Ebrahim, Howard, Kopelman, Sharief, & Williams, 2002). Multiple experiments have revealed that human narcolepsy is caused by orexin deficiency (Tsujino & Sakurai, 2013). Narcolepsy is characterized by the inability to maintain a vigilant state, a pathological intrusion of non-rapid eye movement (NREM) and/or rapid eye movement (REM) sleep into wakefulness, and frequent transitions between states of sleep and wakefulness. Human narcolepsy patients experience excessive daytime sleepiness, manifested particularly as attacks of falling asleep at inappropriate times. They often suffer from cataplexy attacks, which are triggered by emotional stimuli and involve the sudden weakening of postural muscle tone. “Short periods of automatic behavior may also occur, a reflection of brief intrusions of sleep (“micro-sleeps”) into the drowsy state” (Ebrahim, Howard, Kopelman, Sharief, & Williams, 2002).

Influence of Soybean Amino Acids on Orexin Neurons

Branched Chain Amino Acids

Studies have shown that dietary amino acids act directly on orexin neurons to modulate membrane excitability. Amino acids stimulate the electrical activity of orexin neurons through the inhibition of KATP channels and activation of system-A amino acid transporter that preferentially transports nonessential amino acids. (Tsuneki et al., 2012) announced that compared to essential amino acids, nonessential amino acids are more potent in activating orexin neurons. (Karnani, Mahesh M 2011) discovered “BCAAs improve both orexin neuron activation and injury-induced sleep disturbance, indicating that BCAAs can restore wakefulness, at least in part, by activating orexinergic neurons.” The amino acids necessary for the excitation of orexin neurons are present in soybeans. The high content of these amino acids in soybeans could increase the firing rate of orexin neurons, potentially improving wakefulness (Lim et al., 2013).

Conclusion

The rich nutrient content of soybeans makes it a potential source for treatment and prevention of chronic diseases. Soybeans contain essential amino acids, which are necessary for the excitement of hypothalamic orexin neurons and subsequently the increase of orexin availability. Orexin neurons provide crucial links between energy balance, reward systems, arousal, and the sleep-wake cycle. Through the consumption of soybeans, the increase of orexin may alter the course of many chronic disorders, such as narcolepsy. Further research on this subject is required for the development of potential dietary interventions that could benefit those affected with disorders that involve the orexin system.

Chart 1 Amino acid content comparison in soybean, corn, wheat, and bean.

	Soybeans, mature seeds, raw (1.0 cup, 186.0g)	**Corn, sweet, white, raw (1.0 cup kernels, 154.0g)**	**Wheat, sprouted (1.0 cup, 108.0g)**	**Beans, black, mature seeds, raw (1.0 cup, 194g)**
Tryptophan	1.099	0.035	0.124	0.497
Threonine	3.285	0.199	0.274	1.763
Isoleucine	3.666	0.199	0.31	1.851
Leucine	6.155	0.536	0.548	3.347
Lysine	5.033	0.211	0.265	2.877
Methionine	1.017	0.103	0.125	0.631
Cystine	1.218	0.04	0.145	0.456
Phenylalanine	3.947	0.231	0.378	2.266
Tyrosine	2.863	0.189	0.297	1.18
Valine	3.774	0.285	0.39	2.192
Arginine	5.865	0.202	0.459	2.594
Histidine	2.04	0.137	0.212	1.166
Alanine	3.562	0.454	0.319	1.756
Aspartic acid	9.508	0.376	0.489	5.069
Glutamic acid	14.646	0.979	2.021	6.39
Glycine	3.497	0.196	0.33	1.635
Proline	4.425	0.45	0.728	1.777
Serine	4.384	0.236	0.368	2.28

(*Nutrient.*2014)

Table I
Nutrition profile of soybeans expressed per 100 g DM

Composition	*Soybean beans*
Complex carbohydrates (g)	21
Simple carbohydrate (g)	9
Stachyose (mg)	3,300
Raffinose (mg)	1,600
Protein (g)	36
Total fat (g)	19
Saturated fat (g)	2.8
Monounsaturated fat (g)	4.4
Polyunsaturated fat (g)	11.2
Insoluble fibre (g)	10
Soluble fibre (g)	7
Calcium (mg)	276
Magnesium (mg)	280
Potassium (mg)	1,797
Iron (mg)	16
Zinc (mg)	4.8

Table II
Amino acid composition of soybean seed (Liu, 1997b)

Amino Acid	*mg/g Protein*
Arginine	77.16
Alanine	40.23
Aspartic acid	68.86
Cystine	25.00
Glutamic acid	190.16
Glycine	36.72
Histidine	34.38
4-Hydroxiproline	1.40
Isoleucine	51.58
Leucine	81.69
Lysine	68.37
Methionine	10.70
Phenylalanine	56.29
Proline	52.91
Serine	54.05
Threonine	41.94
Tryptophan	12.73
Tyrosine	41.55
Valine	41.55

(Mateos-Aparicio et al., 2008)

Table III. Factors that influence orexin neurons of orexin

Excitation	Receptor involved
Glutamate	AMPAR, NMDAR mGluRs
Acetylcholine (muscarinic) (27%)	M3
Orexin	OX_2R
Ghrelin	GHSR
Cholecystokinin	CCKA
Neurotensin	NTSR2 (unpublished data)
Vasopressin	V1a
Oxytocin	V1a
Glucagon-like peptide 1	ND
Corticotropin-releasing factor	CRFR1
Thyrotropin-releasing hormone	TRH1
BRS3 agonist	BRS3
ATP	P2X
H^+	ASIC1a
CO_2	ND
Mixture of amino acids	System-A amino acid transporters
INHIBITION	
Glucose	Unknown
GABA	$GABA_A$, $GABA_B$
Glycine	Glycine receptor
Serotonin	$5HT_{1A}$
Noradrenaline	α_2
Dopamine	α_2
Acetylcholine (muscarinic) (6%)	ND
Neuropeptide Y	Y_1
Enkephalin	μ opioid-R
Nociceptin	NOPR
Leptin	OBR
Adenosine	A_1
BRS3 agonist (indirect)	BRS3

(Tsujino & Sakurai, 2013)

Table IV. Amino acid composition

Amino acid composition:

Ala	(A)	20	15.3%
Arg	(R)	11	8.4%
Asn	(N)	3	2.3%
Asp	(D)	1	0.8%
Cys	(C)	6	4.6%
Gln	(Q)	6	4.6%
Glu	(E)	2	1.5%
Gly	(G)	16	12.2%
His	(H)	3	2.3%
Ile	(I)	3	2.3%
Leu	(L)	23	17.6%
Lys	(K)	3	2.3%
Met	(M)	2	1.5%
Phe	(F)	0	0.0%
Pro	(P)	12	9.2%
Ser	(S)	10	7.6%
Thr	(T)	5	3.8%
Trp	(W)	1	0.8%
Tyr	(Y)	1	0.8%
Val	(V)	3	2.3%
Pyl	(O)	0	0.0%
Sec	(U)	0	0.0%

(*Protein*.2014)

Figure 1 Schematic representation of inputs and outputs of orexin

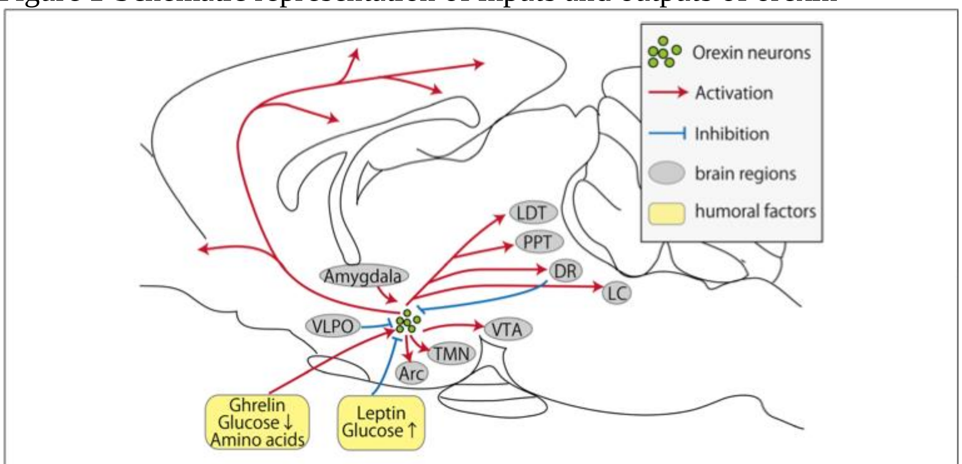
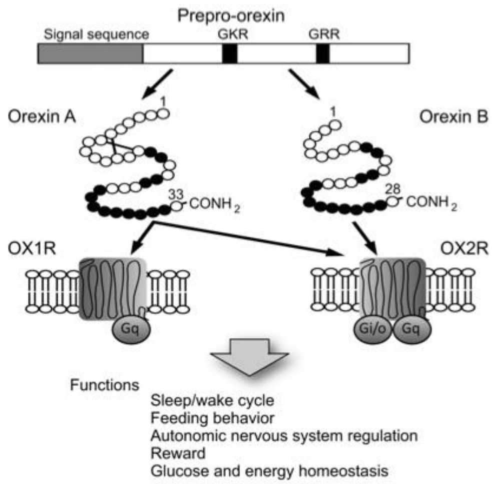


FIGURE 1 | Schematic representation of inputs and outputs of orexin neurons. Orexin neurons are found only in the lateral hypothalamic area but project throughout the entire central nervous system. Red arrows show excitatory projections, while blue lines show inhibitory projections.

Abbreviations: Arc, arcuate nucleus; DR, dorsal raphe nucleus; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; PPT, pedunculopontine tegmental nucleus; TMN, tuberomammillary nucleus; VTA, ventral tegmental area; VLPO, ventrolateral preoptic nucleus.

(Inutsuka & Yamanaka, 2013)

Figure 2 Physiological functions of hypothalamic orexin system



Physiological functions of hypothalamic orexin system
Orexin A and orexin B are neuropeptides generated from a common precursor peptide, prepro-orexin. Closed circles indicate identical amino acid sequence between orexin A and orexin B. Only orexin A possesses disulfide bonds stabilizing the tertiary structure. Orexin A is a high-affinity ligand for both orexin 1 receptor (OX1R) and orexin 2 receptor (OX2R), whereas orexin B is a specific high-affinity ligand for OX2R. The orexin system plays important roles in the regulation of arousal, feeding behavior, autonomic nervous system, reward system, and glucose/energy homeostasis.

(Tsuneki et al., 2012)

Figure 3 Amino acid sequence of orexin

```
        10         20         30         40         50         60
MNLPSTKVSW AAVTLLLLLL LLPPALLSSG AAAQPLPDCC RQKTCSCRLY ELLHGAGNHA

        70         80         90        100        110        120
AGILTLGKRR SGPPGLQGRL QRLLQASGNH AAGILTMGRR AGAEPAPRPC LGRRCSAPAA

       130
ASVAPGGQSG I
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(*O43612(OREX_HUMAN)*.2014)

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Timetable for Completing the Research Project

Preliminary study and reading	Monday, June 2
Begin intensive reading	Friday, June 6
Begin writing Proposal Part I	Friday, June 13
Turn in draft of Proposal Part I to RTL	Thursday, June 19
Begin data collection	Friday, June 20
Re-write Proposal Part I	Monday, June 23
Submit Proposal Part I to Graduate College	Thursday, June 26
Begin writing Final Proposal	Monday, June 30
Prepare for Oral Presentations	Monday, July 7
Turn in draft of Final Proposal to RTL	Thursday, July 10
Present Research at Illinois Summer Research Symposium	Thursday, July 24
End data collection	Monday, July 28
Submit Final Paper to Graduate College	Thursday, July 31