Int. J. Morphol.,
43(6):1833-1842, 2025.

Beyond the Sympathetic and Parasympathetic Nerves; the Time has Come to Classify the Autonomic System Based on Adrenergic or Cholinergic Transmission: What is Happening in the Nervous System?

Más Allá de los Nervios Simpáticos y Parasimpáticos, ha Llegado el Momento de Clasificar el Sistema Autónomo en Función de la Transmisión Adrenérgica o Colinérgica: ¿Qué está Sucediendo en el Sistema Nervioso?

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ZHAO, W-X.; PANG, M. & SHI, Z-Z. Beyond the sympathetic and parasympathetic nerves; the time has come to classify the autonomic system based on adrenergic or cholinergic transmission: What is happening in the nervous system? *Int. J. Morphol., 43(6)*:1833-1842, 2025.

SUMMARY: Horner syndrome (HS) is highlighted by a lack of sympathetic signaling due to the lesion in the upper or lower motor neurons. One of the manifestations of HS could be eye displacement due to the denervation of capsulopalpebral fascia, Müller's, and Kakizaki's muscles. In this study, the lack of a sympathetic system on lipolysis was evaluated. The interaction of the ligands to the sympathetic and parasympathetic receptors was performed using Autodock Vina and ClusPro. The molecular signaling pathway involved in the sympathetic and parasympathetic pathways was analyzed by STRING. Magnetic resonance spectroscopy (MRS) was utilized to measure the neurochemistry of locus coeruleus (LC). The results of the MRS suggested an increase in the LC activity. There was an increase in the lactate value of LC, while there was a decrease in the GABA and choline levels in the affected side in the HS patients. The result of STRING, and molecular docking revealed the involvement of the PACAP and beta 3 adrenergic receptor in the lipolysis. Moreover, the involvement of the PACAP in the activation of the alpha 2 adrenergic receptor suggested its regulatory effect in inducing or inhibiting lipolysis. In the HS the sympathetic system is disrupted, while the parasympathetic system remains intact. The parasympathetic system could control lipolysis through PACAP secretion. This phenomenon is suggested to influence the eye displacement observed in some HS patients.

KEY WORDS: Locus coeruleus; PAG; PACAP; Autonomic nervous system.

INTRODUCTION

The autonomic nervous system (ANS), a key component of the peripheral nervous system, plays an important role in regulating vital involuntary physiological functions in the body, including blood pressure, heart rate, arousal, respiration and digestion, through the development of an intricate network of nerves. Anatomically, it is classified into three distinct subsystems: sympathetic, parasympathetic, and enteric (Kiernan & Barr, 2009).

The sympathetic and parasympathetic systems functionally contain both afferent and efferent fibers that transmit sensory input and motor output, respectively, to the higher brain centers. The opposing but balanced functioning between these two systems causes homeostasis (Karemaker, 2017).

Stimulation of sympathetic system leads to a state called the “fight or flight” response, while activation of the parasympathetic system associated with the “rest and digest” response (Koopman *et al*., 2011). Generally, both the sympathetic and parasympathetic motor pathways contain of a two-neuron sets: a preganglionic (first-order) neuron whose cell body is located in the central nervous system (CNS) and a postganglionic (second-order) neuron whose cell body is outside the CNS and innervates target tissues (Sternini, 1997; Karemaker, 2017). Both systems to

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Received: 2024-11-02 Accepted: 2025-01-21

communicate with target organs and cause specific responses use neurotransmitters. The preganglionic neurons of both the sympathetic and parasympathetic systems use the same neurotransmitter, acetylcholine (ACh), on nicotinic receptors on target tissues. Postganglionic sympathetic neurons primarily use epinephrine and norepinephrine as their transmitter which trigrred a or b adernergeic receptors, while postganglionic parasympathetic neurons use ACh to affect their muscarinic receptors on target effectors (McConalogue & Furness, 1994; Sternini, 1997; Koopman *et al*., 2011).

Due to the wide distribution of the ANS, especially the sympathetic system, and the vital role of this system in the homeostasis of the body, damage to this system is associated with various symptoms and complications due to receives dual innervation from both the sympathetic and parasympathetic systems.

Horner syndrome (HS) is a mild, rare sympathetic nerve supply disorder with the classical manifestation of pupillary miosis, ipsilateral ptosis, facial anhidrosis, and apparent enophthalmos (Rustamzadeh *et al*., 2023a). Enophtalmos could occur as a result of traumatic (orbital fracture, superior cervical ganglion (SCG) sympathectomy, or carotid artery injury) or non-traumatic events (drug abuse, inflammatory diseases, or neurocristopathy which refers to failure in normal neural crest cells migration) (Martin, 2018; Cohen *et al*., 2020). The enophthalmos sign in HS due to the sympathetic pathway injury, including the surrounding plexus of the internal carotid artery (SPICA) which innervates Müller's muscle and the surrounding plexus of the external carotid artery (SPECA) which innervates capsulopalpebral fascia and Kakizaki's muscle, is reported as a result of upper and lower lid ptosis (Codner & Hanna, 2007; Hwang, 2010).

HS could occur as the presence of a lesion in first, second, or third-order neurons nominated as central, preganglionic, and postganglionic regions, respectively. The posterolateral hypothalamus is the location for sympathetic first-order neurons. Locus coeruleus (LC) nerves extend to the paraventricular nucleus (PVN) of the hypothalamus, which controls both parasympathetic and sympathetic preganglionic neurons. Moreover, dendrites of the ventromedial side of the peri-LC receive impulses from ventrolateral periaqueductal grey matter (PAG). The PVN fibers projected to the C8-T1 in the spinal cord are located in the intermediolateral gray column (IML), in which the ciliospinal center of Budge and Waller could be found. Subsequently, nerve fibers extended from the ciliospinal center to the superior cervical ganglion (SCG) making the second-order neurons. The third-order neurons originate from SCG to innervate the iris dilator muscle through two long ciliary nerves (Szabadi, 2013; Kanagalingam & Miller, 2015).

In the absence of sympathetic signals, only parasympathic nerve impulses were present in the affected eye through traveling along the carotid branches (Suzuki & Hardebo, 1993; Bleys *et al*., 2001). Parasympathetic neurons produce pituitary adenylate cyclase-activating peptide (PACAP) and nitric oxide as co-transmitters and vasodilators (Bevan *et al*., 1984). PACAP triggers lipolysis by activating cyclic adenosine monophosphate (cAMP) leading to hormone-sensitive lipase phosphorylation (Holm *et al*., 1997).

In this study the alternative autonomic pathways acting in the absence of sympathetic activity affected eye displacement in the HS were evaluated.

MATERIAL AND METHOD

Study design and participants

In the present case-contrl study, the authors assessed bioinformatics and neuroimaging studies on 13 participants in two groups: I) Patients with Horner syndrome (HS; n = 5) and age-matched healthy controls (HC; n = 8) as the control group.

In the Horner group, patients were recruited. HS was diagnosed based on definite clinical history, ipsilateral miosis, ipsilateral dilation lag, and ptosis, a positive reaction after 0.5 % apraclonidine test, by two expert neuro-ophthalmologists.

Patients who had clinical manifestations of HS for at least 6 months without a history of other eye disorders, neurological diseases and taking psychoactive substances were included in the study. On the other hand, patients diagnosed with disease that influence the eye displacement or mimic HS symptoms such as microphthalmos, orbital fracture, anisometropia, phthisis bulbi, facial hemiatrophy, neurofibromatosis, arthrogryposis, Duane's syndrome, metastatic scirrhous other causes of anisocoria or ptosis, and orbital carcinoma or other malignant tumor were excluded from the study (Morales *et al*., 2000; Chen *et al*., 2006).

The participants of the control group were selected from volunteers who had no history of underlying diseases, neurological disorders, substance use disorders, head trauma, eye trauma, eye movments disorders, and anisocoria.

Neuroimaging Study

To assesses metabolite profiles in the desired regions the two-dimensional multi-voxel magnetic resonance spectroscopy (2D MRS) with point-resolved spectroscopy (PRESS) with automated shim and water suppression using a 1.5 T GE Signa Excite scanner were utilized for this study. The Repetition Time (TR) and time to echo (TE) were set as 1500 ms and 144 ms, respectively. Field-of-view (FOV) in this study was adjusted to 8 cm, matrix size= 9 ¥ 13, data points= 1024, and scan time = 7 min and 25 s). There was an 18¥9¥18 phase encoding matrices along with a 10 mm section thickness. The place of the voxels of interest (VOIs) was set over LC and PAG. The values derived from MRS were normalized with the value of N-acetyl aspartate (NAA) (Rustamzadeh *et al*., 2023b).

Receptor structure preparation and generating grid box

All receptors' files were obtained from the RCSB PDB database (https://www.rcsb.org) (Table I). All the selected receptors were encoded by Homo sapiens genes. Protein files were prepared by MGLTools and AutoDock Tool 4.2 (Sanner, 1999; Morris *et al*., 2009) as follows: automatically the bonds orders were assigned, and systemically atoms were adjusted to the AutoDock atoms types. The receptor structure was cleared from water molecules. Polar hydrogen and Gasteiger-Marsili charges were added to the molecules. Finally, the receptor files were saved in PDBQT format for docking.

Autodock Tool 4.2 was utilized to generate the size of the grid box (Table I). Alpha1B, alpha2A, and beta 3 adrenergic receptors, as well as the M3 muscarinic acetylcholine receptor grid box, were determined based on their agonist and antagonist Moreover, the extracellular domain of the nicotinic receptor was selected as the grid box for docking.

Ligand preparation

To obtain the 3D structure of the norepinephrine (PubChem ID: 439260) and acetylcholine (PubChem ID: 187), the PubChem database was utilized. The pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) structure was obtained from its complex with PAC1 receptor in the RCSB PDB database (PDB ID: 6M1I). Ligands were downloaded in SDF or PDB format. After conversion to proper format through openbabel (O'Boyle *et al*., 2011) (https://openbabel.org/), ligands were modified for molecular docking. The preparation of PACAP was the same as receptor preparation, while norepinephrine and acetylcholine were prepared by applying Gasteiger charges, merging non-polar hydrogen bonds, and setting TORSDOF.

Molecular docking

Autodock Vina was utilized to perform molecular docking for norepinephrine and acetylcholine (Trott & Olson, 2010). The energy range and exhaustiveness were set at four and eight as a default respectively. To exclude the false positive responses (Dahlin *et al*., 2015), ligands were evaluated by false positive remover software (http://cbligand.org/PAINS/search_struct.php) to exclude pan assay interference (PAINS) compounds.

The 3D structure of bound complexes was visualized using Mgltool, Discovery Studio, and AutoDock Tools 4.2. More detailed information on amino acid bonds was determined using LigPlot+ software (Laskowski & Swindells, 2011).

Protein-protein docking was performed by ClusPro server (Vajda *et al*., 2017; Desta *et al*., 2020). The formula of protein-protein docking Etotal was calculated with the following formula (Kozakov *et al*., 2017):

$$E=0.40E_{rep}+(-0.40E_{att})+600E_{elec}+1.00E_{DARS}$$

Clustering analysis

The protein interactions were evaluated using Cytoscape software (https://www.cytoscape.org) (Shannon *et al*., 2003). Protein interaction data was obtained from the STRING database consisting of proteins' interaction along with calculating string text-mining, string score, and coexpression (Szklarczyk *et al*., 2015). Finally, the Betweenness centrality was calculated using Cytoscape software.

Table I. Receptors PDB ID and the information of the grid box size.

Proteins	**PDB ID**	**Grid points**			**Grid**	**Center points**		
		X (Å)	Y (Å)	Z (Å)	**Spacing (Å)**	X	Y	Z
Alpha1B adrenergic receptor	7B6W	12	10	22	1	1.888	26.591	-8.963
Alpha2A adrenergic receptor	6KUX	10	10	12	1	-3.412	-10.454	-19.486
M3 muscarinic acetylcholine receptor	5ZHP	20	18	18	1	5.659	-55.446	151.105
Nicotinic Acetylcholine Receptor	2BG9	80	72	54	1	62.734	63.906	142.237
Beta-3 adrenergic receptor	7DH5	8	10	16	1	77.346	72.793	122.962

RESULTS

In this study, the molecular mechanism and signaling pathways in the parasympathetic neurons were analyzed to evaluate the potential of lipolysis responsible for eye displacement. Finally, neurotransmitter alternation in controlling centers was evaluated through MRS.

Norepinephrine, Acetylcholine, and PACAP interaction

To define the interaction of the norepinephrine, acetylcholine, and PACAP with the receptors involved in the sympathetic and parasympathetic pathway of lypolysis Autodock Vina and ClusPro server were utilized, and the binding affinity and weighted score were calculated (Table II). The results of comparing the receptor's binding site and its key amino acids involved in the receptor activating illustrated no common receptor between norepinephrine and acetylcholine. Although acetylcholine could attach to the binding site of the noradrenergic receptors, its significantly lower binding affinity and less amino acid interaction between acetylcholine and receptor, activation of adrenoreceptors through acetylcholine has a low probability to occur. Similarly, for the acetylcholine receptors (M3 muscarinic and nicotinic receptors), norepinephrine tended to attach to the areas of the receptor which is far from its binding site, therefore, its activation through norepinephrine has less probability. Despite norepinephrine's higher binding affinity in the acetylcholine receptors binding site, by adjusting the grid box for blind docking, norepinephrine had a higher tendency to interact with the amino acids that are far from the receptor binding site.

The weighted score of PACAP and Pituitary adenylate cyclase-activating polypeptide type I (PAC1) receptor was calculated as -1595.5 to compare the binding affinity of PACAP with other receptors. The result of ClusPro illustrated that PACAP had different binding sites in binding with acetylcholine and norepinephrine receptors, except for the alpha 2 noradrenergic receptor (Fig. 1). Although, the weighted score for the binding of PACAP and alpha 2 noradrenergic receptor is less than PAC1 receptor (-1000.4 compared to -1595.5), similar amino acids were involved in the binding of PACAP and norepinephrine (Fig. 2). This result suggested that alpha 2 noradrenergic receptors could activate through PACAP and norepinephrine.

Table II. The result of molecular docking of the norepinephrine and acetylcholine in Autodock Vina, and PCAP in the Hex software.

	Alpha1-noradrenergic receptor	**Alpha2-noradrenergic receptor**	**M3 Muscarinic receptor**	**Nicotinic receptor**	**Beta-3 adrenergic receptor**
Norepinephrine	-5.4 kcal/mol	-5.9 kcal/mol	-4.6 kcal/mol	-6.2 kcal/mol	-6.2 kcal/mol
Acetylcholine	-4 kcal/mol	-3.4 kcal/mol	-3.3 kcal/mol	-4 kcal/mol	-4.5 kcal/mol
PACAP (weighted score)	-1109.6	-1000.4	-1078.7	-1165.9	-959.1

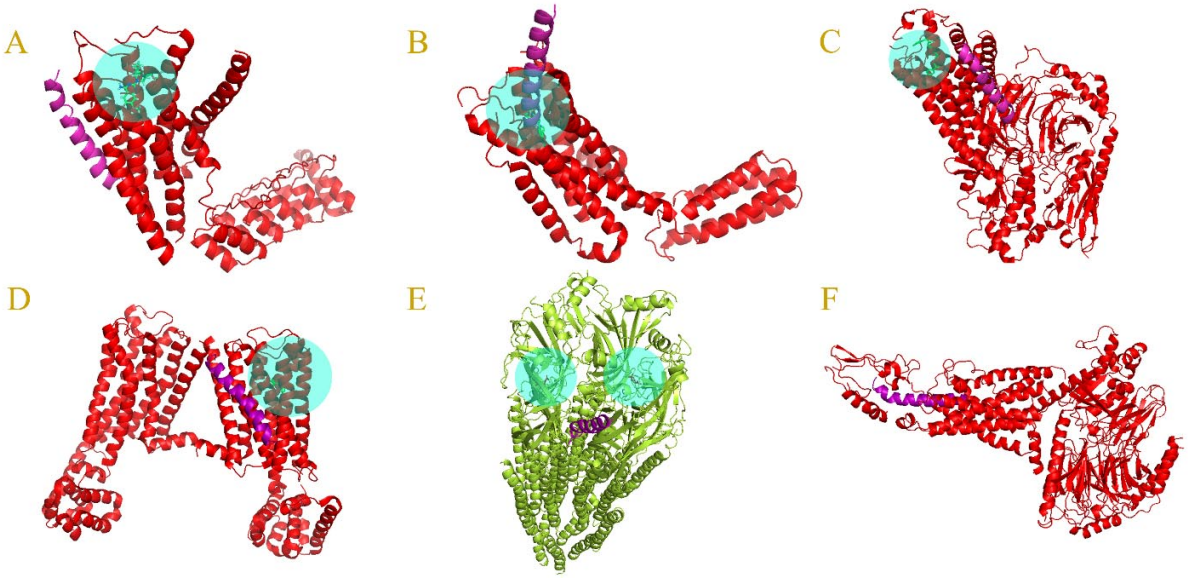

Fig. 1. A) the acetylcholine interaction with alpha1-noradrenergic receptor B) the norepinephrine interaction with alpha2-noradrenergic receptor C) PACAP interaction with beta 3 adrenergic receptor D) PACAP interaction with M3 muscarinic receptor E) PACAP interaction with nicotinic receptor F) PACAP interaction with PAC1 receptor. PACAP and acetylcholine/ norepinephrine binding sites showed in purple and cyan, respectively.

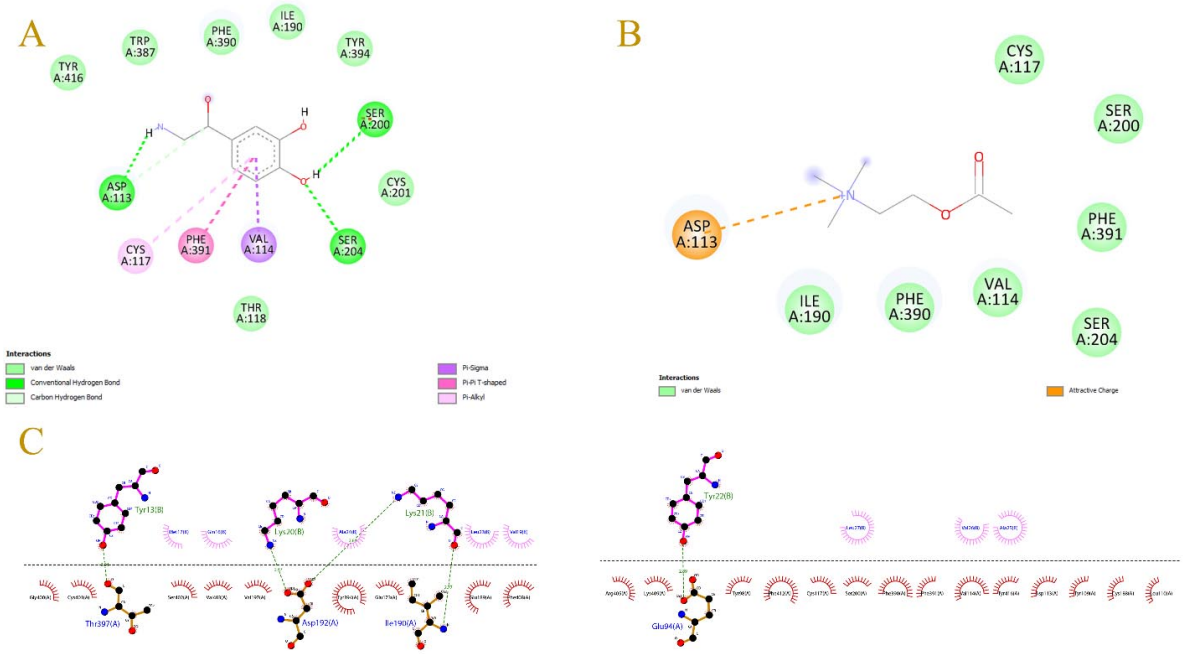

Fig. 2. A) Norepinephrine binding with alpha 2-noradrenergic receptor B) Acetylcholine binding with alpha 2-noradrenergic receptor C) PACAP interaction with alpha2-noradrenergic receptor.

Lipolysis signaling pathway

The molecular signaling pathway of the lipolysis triggered by the sympathetic system has been evaluated through the STRING database. The highest sting score is dedicated to the interaction of PACAP and its receptor named PAC1, Perilipin-1, and hormone-sensitive lipase, as well as protein kinase A, hormone-sensitive lipase, and Perilipin-1. The coexpression value of the PACAP, PAC1, Adenylate cyclase type 2, and protein kinase A indicated that the signaling pathway of the lipolysis could be initiated by the release of PACAP (Table III). Analyzing retrieved

Table III. Protein-protein interaction calculated by STRING and Cytoscape.

Interaction 1	Interaction 2	String Score	String Text Minning	Coexpression	Edge Betweenness	Experimental interaction score
ADCYAP1	ADCYAP1R1	0.999	0.999	0.084	8	0.94
LIPE	PLIN1	0.999	0.996	0.31	2	0.586
LIPE	PRKACB	0.989	0.898		18	
PLIN1	PRKACB	0.96	0.613	0.063	18	0.05
ADRB3	LIPE	0.833	0.826	0.082	2	
ADCY2	PRKACB	0.781	0.364		54	0.096
ADCYAP1	PRKACB	0.629	0.622	0.062	8	
ADRB3	PLIN1	0.614	0.602	0.067	2	0.045
ADRB3	PRKACB	0.604	0.595		18	0.063
ADRA1B	GRK5	0.602	0.19	0.042	22	0.097
ADRA2A	GRK5	0.598	0.563	0.064	32	0.097
ADCY2	ADRA2A	0.539	0.115	0.043	70	
CHRM3	GRK5	0.519	0.079		8	
ADCY2	ADCYAP1	0.496	0.476	0.079	18	
ADRA2A	CHRM3	0.419	0.403	0.067	32	
ADCYAP1R1	PRKACB	0.408	0.395	0.062	14	
CHRM3	CHRNA1	0.405	0.405		22	

ADCY2: Adenylate cyclase type 2; ADCYAP1: Pituitary adenylate cyclase-activating polypeptide; ADCYAP1R1: Pituitary adenylate cyclase-activating polypeptide type I receptor; ADRA1B: Alpha-1B adrenergic receptor; GRK5: G protein-coupled receptor kinase 5; ADRA2A: Alpha-2A adrenergic receptor; CHRM3: Muscarinic acetylcholine receptor M3; CHRNA1: Acetylcholine receptor subunit alpha; GRK5: G protein-coupled receptor kinase 5; LIPE: Hormone-sensitive lipase; PLIN1: Perilipin-1; ADRB3: Beta-3 adrenergic receptor.

enriched publications of the STRING network revealed that the lipolysis and thermogenesis pathway with the involvement of PACAP, PAC1, and beta 3 adrenoreceptor had the lowest false discovery rate (FDR) (Table IV).

Two pathways of lipolysis mediated by beta 3 adrenoreceptor and PAC1 receptor were mediated by protein kinase A, leading to the activation of hormone-sensitive lipase (LIPE) and Perilipin-1 (PLIN1). There was another pathway mediated by alpha 2A adrenoreceptor and G protein-coupled receptor kinase 5 (GRK5) (Fig. 3).

Table IV. Retrieved enriched publication of the STRING network.

Description	FDR value	Genes	P-value	PMID
Adipose Tissue Expression of PAC AP, VIP, and Their Receptors in Response to Cold Stress.	1.40E-04	LIPE|ADRB3|ADCYAP1| ADCYAP1R1	1.87E-10	29982965 *(Cline et al., 2019)*
Adrenergic-Independent Signaling via CHRNA2 Regulates Beige Fat Activation.	0.0014	LIPE|ADRA2A|ADRA1B| ADRB3| CHRNA2	4.59E-09	32533922 (Jun *et al*., 2020)
Neuropeptide PACAP promotes sweat secretion.	0.0059	CHRM3|ADCYAP1| ADCYAP1R1	4.91E-08	28244081 (Cui & Schlessinger, 2017)
The role of genes involved in lipolysis on weight loss program in overweight and obese individuals.	0.0091	LIPE|PLIN1|ADRB3	9.61E-08	26388665 *(Luglio et al., 2015)*
Electroconvulsive seizures lead to lipolytic-induced gene expression changes in mediobasal hypothalamus and decreased white adipose tissue mass.	0.01	LIPE|ADRB3|ADCYAP1	1.17E-07	33426813 (Takefusa *et al*., 2021)
Whole-exome sequencing identifies ADRA2A mutation in atypical familial partial lipodystrophy.	0.0106	LIPE|ADRA2A|PLIN1	1.40E-07	27376152 (Garg *et al*., 2016)

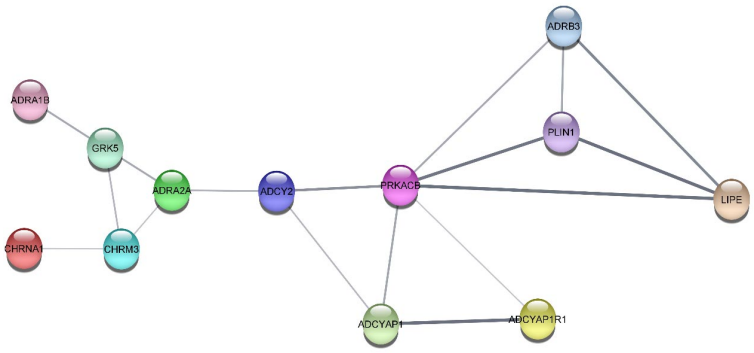

Fig. 3. Protein interaction presented by Cytoscape software. The line thickness is based on the STRING score in the STRING. ADCY2: Adenylate cyclase type 2; ADCYAP1: Pituitary adenylate cyclase-activating polypeptide; ADCYAP1R1: Pituitary adenylate cyclase-activating polypeptide type I receptor; ADRA1B: Alpha-1B adrenergic receptor; GRK5: G protein-coupled receptor kinase 5; ADRA2A: Alpha-2A adrenergic receptor; CHRM3: Muscarinic acetylcholine receptor M3; CHRNA1: Acetylcholine receptor subunit alpha; GRK5: G protein-coupled receptor kinase 5; LIPE: Hormone-sensitive lipase; PLIN1: Perilipin-1; ADRB3: Beta-3 adrenergic receptor.

Locus coeruleus activity measures

To measure the level of acetylcholine through MRS, the level of choline has been measured, due to the metabolism cycle of the acetylcholine which is synthesized from acetyl coenzyme A (from glucose metabolism pathway) and choline. In the MRS analysis, there was a decrease in the level of choline on the affected side manifested by HS symptoms (Table V). This result indicated that the LC increase its inhibitory effect on the parasympathetic fibers of Edinger Westphal (EW) nucleus. Besides, the level of the GABA on the affected side was decreased (Fig. 4). This fact suggested a decrease in the inhibitory input to the LC. From this value, it can be perceived that the activity of LC increased.

Finally, in the metabolism of phenylalanine, there are two pathways; one with the production of phenyl-lactate and phenylpyruvate and the other with the production of norepinephrine through the involvement of tyrosine, L-DOPA, and dopamine. The MRS results on the level of lactate indicated an increase in the lactate level which suggested a decrease in production of norepinephrine (Fig. 4). Nevertheless, lactate is the marker of anaerobic metabolism, and axonal injury which makes it hard to predict what exactly happened to the synaptic activity in the LC.

Table V. The metabolite and metabolite ratios profiles of the LC and PAG in patients with HS and HC.

MRS markers (metabolites)	**LC**			**PAG**		
	HS	**HC**	*p* value	**HS**	**HC**	*p* value
	(n= 8)	**(n= 8)**		**(n= 8)**	**(n= 8)**	
Cho	1.30 ± 0.13	1.85 ± 0.27	0.0001	1.11 ± 0.33	1.67 ± 0.48	0.01
GABA	0.38 ± 0.21	0.51 ± 0.07	0.03	0.25 ± 0.06	0.42 ± 0.17	0.01
Cho/Cr	0.533 ± 0.21	0.397 ± 0.35	0.01	0.296 ± 0.74	0.233 ± 0.48	0.02
mI/Cr	0.78 ± 0.15	0.39 ± 0.23	0.001	1.29 ± 0.20	1.01 ± 0.18	0.01

Data are represented as mean ± SD; Unit of the metabolite concentrations is mM. MRS: Magnetic resonance spectroscopy; LC: Locus coeruleus; PAG: Periaqueductal Gray; HS: Horner syndrome; HC: Healthy control; Cho: Choline; GABA: Gamma-aminobutyric acid; mI: Myo-inositol.

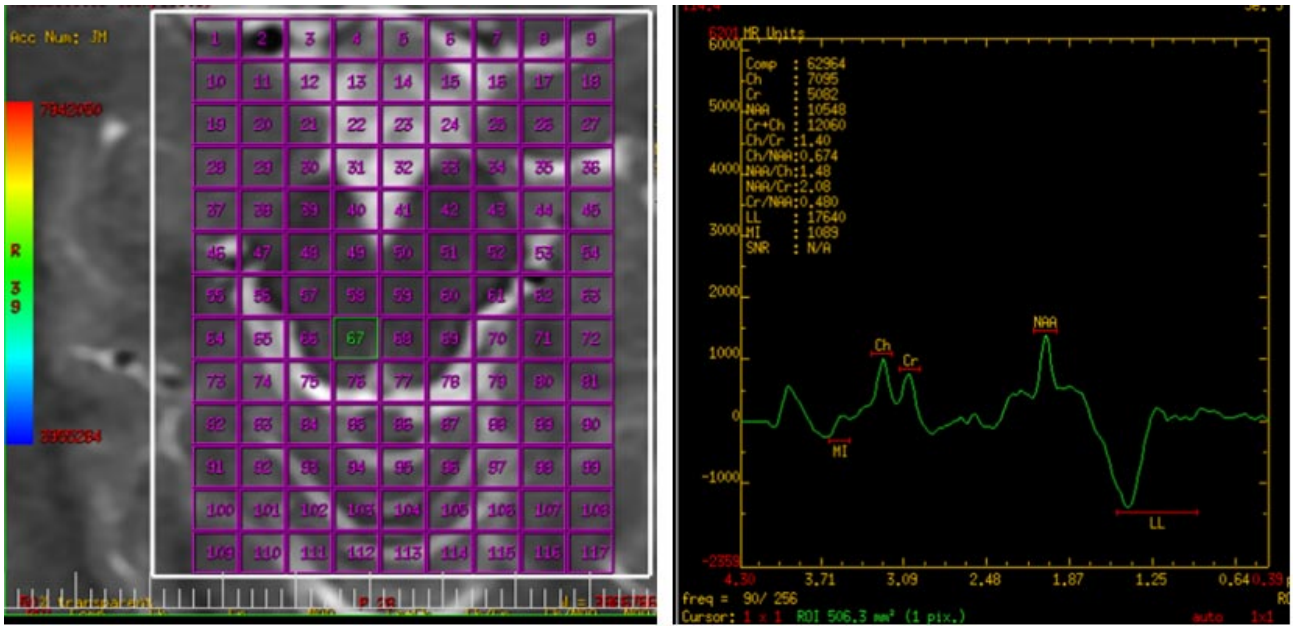

Fig. 4. MRS finding in the activity of LC in the HS patients.

Periaqueductal grey matter activity measures

The result regarding metabolite profiles in PAG showed a significant deacrese of Cho and GABA in HS compared to HC. In addition, Cho/Cr and mI/Cr metabolite ratios were significantly higher than the controls ($p < 0.05$), (Table V).

DISCUSSION

In this study, in-silico analyses were performed to evaluate the binding affinity of the norepinephrine, acetylcholine, and PACAP as the ligands acting on alpha 1 and 2 noradrenergic receptor, M3 muscarinic receptor, and nicotinic receptor. Moreover, the interaction of the proteins involved in the signaling pathway triggered by norepinephrine, acetylcholine, and PACAP in activating lipases was determined through the STRING database. Eventually, the LC activity was measured in both the affected and nonaffected sides through MRS.

The excitatory effect of LC fibers on IML mediates by norepinephrine and alpha1-adrenoreceptor. IML sends impulses to SCG with involvement of acetylcholine neurotransmitter. Subsequently, SCG acts on the dilator pupillae through activation of alpha1-adrenoreceptor through norepinephrine. In addition, PVN could directly activate IML through vasopressin. Besides, LC has an inhibitory effect on the EW nucleus through norepinephrine and alpha2-adrenoreceptors. EW has parasympathetic fibers with acetylcholine neurotransmitters activated ganglion ciliare (GC). GC fibers innervated sphincter pupillae muscles which contract the pupile (Szabadi, 2012). LC control the dilation of the pupils with both sympathetic (SCG) and parasympathetic pathway (EW). In the study conducted by Liu *et al.* (2017), based on the frequency of the LC activation, the ipsilateral or contralateral superior cervical ganglionectomy led to the ipsilaterally sympathetic and parasympathetic effect of LC, while the contralateral

parasympathetic effect of LC on pupil size. Based on the MRS finding, the choline level is decreased which indicates the inhibitory effect of the LC on the production of acetylcholine in the EW nucleus, due to an increase in LC activity.

One manifestation of multiple sclerosis (MS) is a reduction in sympathetic activity due to LC damage, in which patients suffer from fatigue and pain in their lifestyle (Niepel *et al*., 2013; Racke *et al*., 2021). Moreover, LC gets impulses from ventrolateral PAG which is stated to be involved in pain modulation, in which its stimulation results in the production of antinociception (Szabadi, 2013). The connection of LC and PVN causes PVN activation through LC in stress conditions leading to neurotransmitters and hormone release (Reyes *et al*., 2005). Based on the MRS finding which indicated an increase in norepinephrine release from LC and overactivity of LC on the damaged side, it is predicted that in traumatic events, in which the sympathetic system is damaged, the PAG and LC feedback increased to compensate for the sympathetic damage.

The oculomotor nucleus which controls the movement of external eye muscles has been reported to express alpha1-adrenoreceptors which are innervated by noradrenergic fibers (Szabadi, 2013). In the oculomotor nucleus, the PACAP and PAC1 were detected and upregulated in traumatic events or motor neuron diseases (Maugeri *et al*., 2020). Based on the STRING results, PACAP secretion and its binding to PAC1 could activate ADCY2 leading to activation of the signaling pathway resulting in lipolysis.

In the lipolysis signaling pathway, alpha 2 adrenergic receptor by G protein interference inhibits lipolysis (Garenc *et al*., 2002). In contrast, PACAP could increase lipolysis in targeted adipose tissue. Moreover, its activity is regulated by insulin in which high concentrations of insulin decrease lipolysis mediated by PACAP (Akesson *et al*., 2003). Besides, the alpha-1 adrenergic receptor is involved in glycogenolysis and smooth muscle contraction (Biazi *et al*., 2018). The STRING results depicted three pathways. The first pathway had an excitatory effect on lipolysis mediated by PACAP. The second pathway had an inhibitory effect on lipolysis mediated by alpha-2 adrenergic receptors. Finally, the third pathway, referred to the smooth muscle contraction is mediated by alpha-1 adrenergic receptor. The result suggested that M3 muscarinic receptors were activated in the parasympathetic fibers (Billington & Penn, 2002) and nicotinic receptors in the sympathetic system (Williams *et al*., 2011) influenced alpha-2 adrenoreceptors activation which inhibited lipolysis. There was another parasympathetic signaling pathway mediated through PACAP resulting in lipolysis. The sympathetic signaling pathway resulting in the activation of the nicotinic receptor with acetylcholine through IML influenced the activity of parasympathetic neurons labeled by M3 muscarinic receptors. In the normal individual whose both sympathetic and parasympathetic system was intact there is a balance between inhibition and activation of lipolysis, whereas, in the HS, in which the sympathetic system is interrupted, the pathway involved alpha-2 adrenergic receptor was absent leading to removal of inhibitory effect of lipolysis.

The sympathetic system had an important role in lipolysis (Zeng *et al*., 2015). A previous study stated that the balance between the b adrenoceptors and alpha-2 adrenergic receptors maintains the balance of the lipolysis (Bartness & Song, 2007). Both b and alpha adrenoreceptors were activated through the sympathetic nervous system (Binder *et al*., 2009). In the absence of a sympathetic system, only the parasympathetic system could influence the volume of adipose tissue through PACAP. Moreover, PACAP is involved in stress responses by acting on the hypothalamus to increase sympathetic output (Cline *et al*., 2019). Our protein docking analysis suggested that PACAP could activate both PAC1 and alpha-2 adrenergic receptors. Mutation in the ADRA2A gene is linked to lipodystrophy. The results of the Garg *et al*. (2016), conducted research in the 3T3-L1 adipocytes human cell line highlighted more cAMP and glycerol production in the case of ADRA2A mutation. Since alpha 2 adrenoreceptor inhibited lipolysis, PACAP could control the white adipose tissue mass. One of the current study limitations was our limited number of participants. Lack of funding source to conduct the project, impeded us from enrolling a sufficient number of patients. Moreover, there is encouragement to perform in vitro and in vivo studies to better justify the role of PACAP in lipolysis. Consequently, it can be perceived that increased LC activity with the interference of ventrolateral PAG due to the sympathectomy stimulation of the oculomotor nucleus activity leads to upregulation in PACAP expression. PACAP dysregulation in the absence of a sympathetic system could induce lipolysis in the oculomotor innervation area. This mechanism is proposed to deteriorate the eye position in some HS patients.

CONCLUSION

In conclusion, the result of the protein-ligand analysis suggested that PACAP could activate both alpha 2 adrenoreceptor and PAC1 receptor. The STRING result highlighted PAC1 and beta 3 adrenergic receptors in activating lipolysis. Finally, the higher activity of LC in the affected side in the HS patients, demonstrated that norepinephrine release from LC and impulses to the oculomotor nucleus innervated eye globe increased.

ZHAO, W-X.; PANG, M. & SHI, Z-Z. Más allá de los nervios simpáticos y parasimpáticos, ha llegado el momento de clasificar el sistema autónomo en función de la transmisión adrenérgica o colinérgica: ¿Qué está sucediendo en el sistema nervioso? *Int. J. Morphol., 43(6)*:1833-1842, 2025.

RESUMEN: El síndrome de Horner (HS) se caracteriza por una falta de señalización simpática debido a la lesión en las neuronas motoras superiores o inferiores. Una de las manifestaciones del HS podría ser el desplazamiento ocular debido a la denervación de la fascia capsulopalpebral, el músculos tarsal superior (de Müller y Kakizaki). En este estudio, se evaluó la falta de un sistema simpático en la lipólisis. La interacción de los ligandos con los receptores simpáticos y parasimpáticos se realizó utilizando Autodock Vina y ClusPro. La vía de señalización molecular involucrada en las vías simpática y parasimpática fue analizada por STRING. La espectroscopía de resonancia magnética (MRS) fue utilizada para medir la neuroquímica del locus coeruleus (LC). Los resultados de la MRS sugirieron un aumento en la actividad del LC. Hubo un aumento en el valor de lactato del LC, mientras que hubo una disminución en los niveles de GABA y colina en el lado afectado en los pacientes con HS. El resultado de STRING y el acoplamiento molecular reveló la participación del PACAP y el receptor adrenérgico beta 3 en la lipólisis. Además, la participación del PACAP en la activación del receptor adrenérgico alfa 2 sugirió su efecto regulador en la inducción o inhibición de la lipólisis. En el HS el sistema simpático está alterado, mientras que el sistema parasimpático permanece intacto. El sistema parasimpático podría controlar la lipólisis a través de la secreción de PACAP. Se sugiere que este fenómeno influye en el desplazamiento ocular observado en algunos pacientes con HS.

PALABRAS CLAVE: Locus coeruleus; PAG; PACAP; Sistema nervioso autónomo.

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