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Fetal autonomic nervous system development and regulation

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Maio, 2023

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Artigo de revisão bibliográfica

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Porto, Maio 2023

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ACKNOWLEDGMENTS | AGRADECIMENTOS

Após todo este percurso não poderia deixar de exprimir a minha gratidão a todos aqueles que contribuíram de alguma forma para a realização deste trabalho.

Gostaria de agradecer ao meu orientador, o Professor Doutor Luís Guedes-Martins, por aceitar fazer parte deste projeto e por todo o apoio incansável, disponibilidade e compreensão demonstrados ao longo de todo o trabalho.

Aos meus pais e irmã, pela motivação e suporte que me permitiu priorizar a minha vida académica, bem como a realização desta dissertação. Não existem palavras suficientes para descrever o quão grata estou por vos ter comigo.

Agradeço aos meus amigos para a vida, pelo apoio incondicional, conselhos e memórias construídas ao longo destes seis anos.

RESUMO

O sistema nervoso autónomo é essencial para assegurar a homeostasia cardiovascular, respiratória e gastrointestinal durante a transição fisiológica fetal para neonatal. O sistema nervoso autónomo inclui duas divisões: o sistema nervoso simpático e o sistema nervoso parassimpático, cujo desenvolvimento requer diferentes fatores de transcrição e proteínas e ocorre de forma assíncrona.

A exposição a fatores como o stress, a desnutrição e os tóxicos durante a gestação pode afetar o desenvolvimento e a regulação do sistema nervoso autónomo, conduzindo a doenças e efeitos secundários a longo prazo. Através destes mecanismos de doença, conseguimos compreender como os fatores de risco presentes numa fase fetal levam ao aparecimento de patologias mais tarde.

O sistema nervoso autónomo é responsável pela regulação da frequência cardíaca, pelo que a atividade do sistema nervoso autónomo pode ser medida através da variabilidade da frequência cardíaca.

Assim, o objetivo desta revisão foi compreender como se processa o desenvolvimento do sistema nervoso autónomo e como alguns fatores alteram o seu desenvolvimento normal, conduzindo a várias patologias mais tarde na vida.

Palavras-chave: fetal, sistema nervoso autónomo, sistema nervoso simpático, sistema nervoso parassimpático, sistema nervoso entérico, variabilidade da frequência cardíaca

ABSTRACT

The autonomic nervous system is essential to ensure cardiovascular, respiratory and gastrointestinal homeostasis during the fetal to neonatal physiological transition. The autonomic nervous system includes two divisions: the sympathetic nervous system and the parasympathetic nervous system, whose development requires different transcription factors and proteins and occurs asynchronously.

Exposure to factors such as stress, malnutrition, and toxicants throughout gestation can affect the development and regulation of the autonomic nervous system, leading to long-term illness and side effects. It is through these disease mechanisms that we are able to understand how risk factors present at the fetal stage can lead to the development of other pathologies at a later stage.

The autonomic nervous system is responsible for regulating heart rate and therefore the activity of the autonomic nervous system can be measured using heart rate variability.

Thus, the objective of this review was to understand how the development of the autonomic nervous system takes place and how some factors affect and alter its normal development, leading to various pathologies later in life.

Keywords: fetal, autonomic nervous system, sympathetic nervous system, parasympathetic nervous system, enteric nervous system, heart rate variability

ABBREVIATIONS LIST

ANS – Autonomic nervous system
BMP – Bone morphogenetic protein
ECG – Electrocardiography
ENS – Enteric nervous system
i.e. - *id est*
MeSH – Medical Subject Headings
NCC – Neural crest cell
PNS – Parasympathetic nervous system
SNS – Sympathetic nervous system

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INTRODUCTION

One of the most important factors in the regulation of the human physiological functions is the autonomic nervous system (ANS), which includes two divisions: the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS).[1–3] However, the sympathetic and parasympathetic division develop asynchronously and therefore their influence on foetal life starts at different periods.[4] Nonetheless, without this regulation, human beings would not be able to cope with both external and internal stressors, leading to various adverse consequences.[5]

The ANS and the heart develop simultaneously, so this ability to regulate begins even before birth.[6] However, since at birth the ANS is not yet fully mature, it is vulnerable to environmental and physiological factors, which influences the development of the fetus.[6,7] This system works both ways, meaning that any abnormal change in intrauterine development can result in altered ANS capabilities.[6,7]

In the newborn, the ANS is essential to ensure cardiovascular, respiratory and gastrointestinal homeostasis during the fetal to neonatal physiology transition.[8,9] The ANS also plays a major role in the support of high cortical functions, which if altered, can lead to the development of neuropsychiatric disorders in childhood.[8,9]

The ANS controls the heart rate, increasing it through the SNS and decreasing it through the PNS.[10] Therefore, ANS activity can be measured by heart rate variability (HRV) using electrocardiography (ECG), however this method is affected by developmental changes in both fetus and infant.[11–15] Since the 1960s, HRV has been used to predict some lethal complications that may occur in the fetus, although it is not the ideal method for identifying fetal well-being.[16,17] It would therefore be important to identify the intervals in the perinatal period that provide the most information regarding ANS, in order to monitor fetuses effectively and ensure safe maternal-fetal care.[4,6]

Exposure to factors such as stress, malnutrition, and toxicants throughout gestation can affect the development and regulation of the ANS, leading to long-term illness and side effects.[10] Something else to take into consideration is the fact that the outcome of this exposure depends not only on the type, but also on the period of gestation in which the exposure occurred.[10] Through these disease mechanisms, we are able to understand how risk factors present at a fetal stage lead to the emergence of other pathologies later on.[10,18–20]

Therefore, the aim of this review was to understand how the normal development of the fetal ANS takes place and also how it is regulated and how it may affect other systems. The purpose of this paper is also to identify and understand why some factors affect the development of ANS and how it can lead to disease later in life.

METHODS

Search methods:

For this review we are using PubMed as the main database for our research, although we intent on using Cochrane Library and Scopus as well. We will do an electronic search, where we will select articles either in English, Portuguese or Spanish, without any other filters.

In order to obtain a greater number of valuable articles, we are going to use the MeSH Database, with the following MeSH terms: "autonomic nervous system" AND "age, fetal", “sympathetic nervous system” AND “age, fetal”, “parasympathetic nervous system” AND “age, fetal” and “enteric nervous system” AND “age, fetal”. PubMed Advanced Search Builder will also be used with words related to "autonomic nervous system" OR “sympathetic nervous system” or “parasympathetic nervous system” OR “enteric nervous system” OR “heart rate variability”, among others. We will also search the reference list of other articles and reviews whose objective is similar to ours for other potentially relevant articles.

Inclusion and Exclusion Criteria:

Our initial research method will be based on the selection of studies that are focused on the topic of fetal autonomic nervous system and on several keywords, such as development, regulation, sympathetic nervous system, parasympathetic nervous system, enteric nervous system and HRV, and others. We will include reviews and observational studies, including cohort, case-control, cross-sectional, case-reports or case-series and decided to exclude commentaries and conference abstracts from this research.

Using these terms there were 805 articles that contained the keywords written above or that were in the reference list of other articles. These searches were repeated several times to obtain the most recent articles that could be included in our review.

Through this first filter, based on the titles and abstracts of each article, a single reviewer selected 306 citations that might contain useful information for this review. After this first selection, we proceeded to read all the articles in their entirety in order to choose the articles that are more directed to the objective of this article, arriving 226 citations in the end (Fig. (1)).

FETAL DEVELOPMENT OF THE SYMPATHETIC NERVOUS SYSTEM

The SNS is generally known for its role in the "fight or flight" response mechanism, but it also plays an essential role in the maintenance of homeostasis during the various activities of an individual's daily life.[21] In order for homeostasis to be maintained efficiently, precise connections must be made between the postganglionic sympathetic neurons and the peripheral organs.[21,22]

The development of the SNS begins in the neural crest, which arises from the lateral margins of the neural plate while invaginating to form the neural tube.[23,24] The neural crest cells (NCCs) of the trunk give rise to sympathetic and sensory neurons, glial satellite cells, Schwann cells, and paraganglion cells.[25] The cells in the dorsal part of the neural tube undergo a transformation from epithelial to mesenchymal characteristics, after which they delaminate and migrate to different target sites.[23,24] The migration of NCCs requires epithelial-mesenchymal transition, which is dependent on Wnt/β-catenin and bone morphogenetic protein (BMP) signaling.[26,27]

Preganglionic and postganglionic sympathetic neurons

The SNS is divided into preganglionic and postganglionic sympathetic neurons, which are anatomically organized in series and linked by synapses.[21] Starting with the preganglionic neurons these reside in an intermediolateral thoracic and lumbar column of the spinal cord and extend their axons to connect to the postganglionic neurons which have two distinct locations.[21] Some are located in the paravertebral ganglia that lie in the bilateral chains along the rostro-caudal axis.[21] These neurons of the paravertebral ganglia send axonal projections to innervate the pupil, the heart, the respiratory system, the blood vessels, and the exocrine glands of the face, the trunk, and the limbs.[21] The other set of neurons are found in the prevertebral ganglia located between the chain and the peripheral tissues, and these are responsible for the innervation of the abdominal, pelvic and perineal organs.[21] The superior cervical ganglion and the stellate ganglion are located more rostrally.[24]

During pre- and postganglionic sympathetic neuron development, hypoxia-inducible factor 1 is essential for developing cardiac sympathetic innervation and maintaining oxygen homeostasis.[24,28–30] Its absence leads to a profound deficit in sympathetic innervation of the heart and disrupts the development of sympathetic ganglia and chromaffin cells.[31]

The superior cervical ganglia are located at the bifurcation of the internal and external carotid arteries, and their axons follow these structures to innervate the tissues of the head and face.[32] A subtype of upper cervical ganglia expresses the EdnrA receptor for endothelin 3, which is selectively produced by the external carotid arteries, causing this subtype of axons to preferentially

follow the external carotid artery in order to reach the salivary glands.[32] The neurons in the superior cervical ganglia are dependent on the growth factor GDNF, Wnt ligands, and Frizzled receptors for their survival and target innervation.[33–36] When the GDNF receptor, called RET, is deleted, there is a significant reduction in sympathetic neurons in the superior cervical ganglia, but no changes at the paravertebral level.[37,38]

The three stages of neural crest cells migration

In the first delamination phase, NCCs migrate ventrally along blood vessels between somites.[39] In the intermediate stage, the cells migrate ventrally across the anterior portion of the somites in the sclerotome.[39] In the final wave, the NCCs move dorsolaterally between the epidermis and the dermomyotome.[39] The early and intermediate phase NCCs give rise to the neurons of the dorsal root ganglia and, more ventrally, to the sympathetic neurons and the chromaffin cells.[23]

Neuregulin 1 and its receptors ErbB2 and ErbB3 are important for the ventral migration of NCCs.[23,40] However, migration to the aorta depends on the induction of both Nrg1 and Cxcl12, the gene encoding SDF-1.[23,40]

Neuropilin 1 and 2 are also present in the NCCs of the ventral pathway, with high levels of Nrp1 and low levels of Nrp2 present in the intermediate stage, giving rise to sympathetic ganglia, while high levels of Nrp1 and Nrp2 give rise to dorsal root ganglia cells.[23,39]

NCCs following the ventral path retain FoxD3 expression, whereas cells following the lateral path, i.e., melanocytic NCCs, lose FoxD3 expression.[41,42] Pre-migratory NCCs express Sox8, Sox9 and Sox10, with Sox10 expression being maintained during migration, as it is critical for survival and differentiation into specific lineages.[43–45] One of the peculiarities of sympathetic neurons is that they have the ability to re-enter the cell cycle, even after the expression of genes that are specific for a particular phenotype.[46–48]

The early cohort of NCCs express the CXCR4 receptor and migrate ventrally in response to their ligand SDF-1/CXCL12, which is present in the vicinity of the dorsal aorta.[49] Cxcr4-negative NCCs give rise to peripheral ganglia.[49]

NCCs near the dorsal aorta differentiate into sympathetic neuroblasts and eventually sympathetic neurons.[39,50] BMPs, particularly BMP2, BMP4, and BMP7, are the main regulators of NCC differentiation.[23] However, NCC differentiation is also regulated by several transcription factors, including Ascl1, Phox2a and Phox2b, Insm1, Hand2, Gata3, Sox4 and Sox11, Isl1 and AP-2.[23] The transcription factor Phox2b also plays a crucial role in the differentiation and survival of sympathetic nerve cells.[51] Loss of Phox2b results in apoptosis of sympathetic nerve precursors even

before they reach the dorsal aorta.[51] In addition, Ascl1 is required for sympathetic axon growth, as loss of Ascl1 also leads to a reduction in sympathetic neuron numbers.[24]

Sympathetic neuroblasts express tyrosine hydroxylase, which is a marker usually associated with mature sympathetic neurons.[31,52] However, despite this expression, sympathetic neuroblasts still undergo proliferation.[31,52]

After reaching the dorsal aorta, sympathetic precursors secrete rostrocaudally to form discrete ganglia, a process dependent on N-cadherin adhesion molecules and EphrinB/EphB signaling.[53]

There is then a second migration in which the mature sympathetic neurons come into contact with the preganglionic sympathetic axons and induce the migration of the nascent sympathetic ganglia to their final location near the ventral root of the spinal cord.[49] This process is mediated by BDNF, which is secreted by preganglionic sympathetic axons and is responsible for activating TrkB receptors on sympathetic neurons.[49] Differentiation of sympathetic neurons is influenced by the signaling of several BMPs secreted by the dorsal aorta and the expression of several transcription factors including Phox2a, Phox2b, Gata2, Gata3, INSM1, Hand2, Ascl1, Sox4, Sox11 and LIM homeodomain protein Islet-1.[54–57]

Sympathetic axons elongation

The mechanism by which axonal specification occurs is not yet well defined, however, it is known that sympathetic neurons extend axons very early in the embryonic stage.[58] Outgrowth of sympathetic axons and sympathetic neuroblast differentiation occurs through signaling by hepatocyte growth factor acting through its tyrosine kinase receptor c-Met.[59–61] Hepatocyte growth factor is therefore required locally for sympathetic axon growth, an essential role during axon development or after neuronal injury.[60]

The axons projecting from the ganglia undergo rapid elongation, and extend using blood vessels as an intermediate path to reach their final target tissues.[37,58] The proximal axons grow close to the arteries due to the forces of attraction created by artemin and neurotrophin 3.[22,62] Artemin is a member of the GDNF family of ligands expressed in smooth muscle cells of the vessels, that uses the RET/GFRalpha3 receptor complex to act as a chemoattractive factor that encourages sympathetic fibers to follow blood vessels as they project toward their final target tissues.[37,63] However, some axons, even in the absence of artemin or neurotrophin 3, still reach their target, suggesting the existence of a possible synergistic effect between these factors or the existence of additional signals. [62,63]

Target innervation

During development, axons of postganglionic sympathetic neurons travel long distances to form connections with various peripheral targets.[21] Sympathetic innervation of the target tissues is mainly controlled by the neurotrophin called nerve growth factor, which is produced by the sympathetic targets, whose innervation density correlates with the amount of nerve growth factor produced by an organ.[64,65] The inactivation of the growth factor or its receptor TrkA results in the loss of sympathetic ganglia.[66,67]

In distal axons, nerve growth factor binds to TrkA receptors to regulate cytoskeletal dynamics through activation of the MAPK and PI-3K-Akt pathways.[68,69] Endocytosis of the TrkA receptors triggered through a calcineurin-dynamin 1 signaling pathway also promotes target growth and innervation.[70] Target innervation by distal axons depends not only on nerve growth factor, but also on local synthesis and lipidation of Rac1 and the presence of Tp53inp2 and Impa1.[71–73]

Not only does nerve growth factor mediate innervation at the distal target site, but it also mediates axonal growth, and when the transcription factors CREB or early growth response are absent, abnormalities in these processes occur.[74,75] Retrograde nerve growth factor signaling in sympathetic neurons induces Wnt5a synthesis which promotes axon branching through Ror1/2 receptor signaling.[33,76,77] Signaling by CD40L promotes innervation of peripheral tissues that express only low levels of nerve growth factor, such as the thymus, because nerve growth factor regulates through negative feedback the activity of CD40L.[78]

In certain end targets, such as the gastrointestinal tract, gonads, and trachea, the absence of nerve growth factor does not seem to have as important an effect as in the other targets, and they continue to receive innervation, some less so, and some, such as the trachea, are not affected at all.[67] In addition, tissue-specific growth factors can promote selective target innervation, such as is the case with brown adipose tissue innervation that is due to the selective expression of calsyntenin 3β, which in turn controls the secretion of growth factor S100b.[79]

The developing heart expresses semaphorin 3A, and its absence leads to adverse consequences on cardiac innervation, but its overexpression leads to reduced cardiac innervation.[80] This is due to semaphorin 3A inhibiting nerve growth factor-induced sprouting and semaphorin 3F suppressing TrkA-mediated MAPK and PI-3K signal transduction.[68,81]

In late stages of development excessive axonal branching occurs at the end targets, which gives rise to a competition for target-derived nerve growth factor that leads to the elimination of the "losing" axon terminals.[82] In sympathetic axons pruning is mediated by p75, suppression of TrkA-dependent trophic signaling, death receptor 6 and increased intracellular calcium.[82–85]

Synaptic connectivity

It is not known precisely how sympathetic axons establish contacts with peripheral targets, however it is thought that there is neurotransmitter release at the sites facing the target cells.[86–88]

In cardiac myocytes, sympathetic neurons establish synaptic contacts with myocytes, contacts that are increased by nerve growth factor.[89,90] The nerve growth factor also increases the synthesis of norepinephrine and potentiates the release of norepinephrine, leading to depolarization of the myocytes and consequently to an increase in heart rate.[89,90]

Preganglionic axons enter the sympathetic ganglia prior to dendrite formation and establish nicotinic cholinergic synapses with the cell bodies of the postganglionic neurons.[58,91,92] The development of synaptic connections between preganglionic and postganglionic sympathetic neurons is regulated through retrograde nerve growth factor signaling, which promotes the secretion of synaptogenic factors, such as BDNF from postganglionic neurons, but also the clustering of postsynaptic components, such as nicotinic acetylcholine receptors. [65,92–94] In addition, activated trkA receptors are required in the postganglionic neurons in order to maintain the synaptic connections with the preganglionic neurons.[65] Sympathetic cholinergic synapses contain supporting proteins PSD93 and PSD95, MAGUK, GKAP/SAPAP and Shank/ProSAP, and are therefore similar to glutaminergic synapses in the central nervous system.[95]

Neuron survival

Retrograde survival signaling is communicated from the dynein-mediated vesicular transport pathway of internalized NGF-TrkA receptor complexes.[96,97] Several types of endosomal organelles may be responsible for this transport, and Rab5-positive early endosomes or Rab7-positive late endosomes have been identified as long-distance transporters.[98,99] In cell bodies, axon-derived Trk endosomes promote survival by signaling facilitated by the endosomal effector coronin 1 and through activation of downstream transcription factors, including CREB.[100–102]

Retrograde nerve growth factor signaling leads to the activation of positive feedback loops, which gives rise, for example, to the transcription of its own TrkA receptor and also leads to the expression of pro-death signals that leads to the death of neighbouring neurons.[100,102,103]

Activation of p75 in distal axons in response to nerve growth factor withdrawal leads to proteolytic cleavage of p75 and long-distance transport of its intracellular domain into cell bodies.[104,105] Dual leucine zipper kinase and c-Jun-N-terminal kinase also signal the initiation of apoptotic signals.[106,107]

Diversity of sympathetic neurons

Sympathetic neurons are very heterogeneous, with the fate of sympathetic neurons beginning in a common noradrenergic-cholinergic sympathetic progenitor.[108]

Differentiation into the cholinergic type occurs due to regulation by the transcription factor TLX3.[108] For the cholinergic gene expression it is also required Ret, which is negatively regulated by HMX1.[65] The cytokines leukaemia inhibitory factor and ciliary neurotrophic factor appear to be involved in the differentiation of neurons for cholinergic properties, however, in the absence of these factors, differentiation still occurs, suggesting that other factors may be involved.[109–111] The noradrenergic type, however, results from regulation by HMX1 transcription factor.[108]

Some neurons acquire cholinergic properties early in life, but some sympathetic noradrenergic neurons change to cholinergic properties upon postnatal innervation of the target tissue.[112–115] This switch requires neurokine signaling via the gp130 receptor.[65]

Several transcription factors interact with each other so that sympathetic neuronal differentiation can occur, including Phox2a and 2b, Gata2 and 3, Hand1, 2 and 3, and Ascl1.[65]

Development of satellite ganglia

Furthermore, a subtype of cells called glial satellite cells surround the cell bodies of sensory, sympathetic, and parasympathetic neurons and perform functions at the level of neuronal activity and morphogenesis.[116]

The transcription factor Sox10, which is expressed in all migrating NCCs is important in determining the fate of glial cells.[117,118] Another marker for the development of glial cells is the brain lipid-binding protein, which is expressed in glial precursors and can also be identified after the maturation of glial cells.[119–121]

Glial cell development occurs simultaneously with preganglionic input and target innervation, so it is possible that retrograde signaling by nerve growth factor is involved in satellite glial cell development. Loss of NGF-TrkA signaling in animal models has led to glial cell loss, but the mechanism by which this occurs is not well understood.[58,66,67,122]

Sympathetic disfunction and disease

Sympathetic dysfunction is associated with several pathologies including peripheral neuropathies, heart failure, hypertension, diabetes mellitus, immune dysfunction, and epithelial

neoplasms and may also contribute to the development of familial dysautonomia, Down's Syndrome, cardiac and metabolic dysfunction.[123–132]

Familial dysautonomia is a type of peripheral neuropathy, caused by mutation in Elp1, that leads to the development of cardiovascular, renal, gastrointestinal, and pulmonary dysfunction.[129,133]

Down's Syndrome is caused by trisomy 21, leading to autonomic dysfunction at the level of heart rate and blood pressure.[134] The loss of sympathetic neurons in this pathology occurs due to excess of the regulator of calcineurin 1, which promotes internalization of TrkA, leading to dysregulation of TrkA signaling.[124]

SNS dysfunction is associated with several pathologies of cardiovascular origin, such as sudden fetal death syndrome, hypertension, myocardial ischemia, and cardiac arrythmias.[125,128,135,136] Alterations in cardiac sympathetic innervation during development can result in heart rate abnormalities, heart failure, or even death, due to overexpression or loss of signaling by semaphorin 3A or endothelin 1.[80,137,138] Nerve growth factor, if at extremely high levels, can lead to cardiac hypertrophy and sudden cardiac death.[139,140] In addition, glial satellite cells also seem to play a role in stimulating sympathetic activity, leading to increased heart rate and force of contraction.[141] In addition, sympathetic innervation contributes to the determination of cardiac size in the adult by regulating the total number of cardiomyocytes.[142] Sympathetic neurons delay cardiomyocyte maturation and cell cycle withdrawal, i.e., regulate the proliferative/hypertrophic transition.[142] Because of this, injury to the SNS during development results in decreased cardiac size, among other cardiac consequences.[142]

The SNS is responsible for the control of the secretion of various hormones, the production of glucose, and the metabolism of glucose and lipids.[143] The presence of sympathetic innervation is necessary for the formation and functional maturation of pancreatic islands of Langerhans.[126] Early disturbances in sympathetic innervation can lead to reduced insulin secretion and lower glucose tolerance, and may be at the root of the development of type 1 diabetes mellitus.[126,144,145]

The sympathetic nerves of the bone marrow are regulators of the mobilization, proliferation and renewal of progenitor cells and hematopoietic stem cells.[146] In acute myeloid leukaemia there is loss of sympathetic neurons, leading to aberrant expansion of progenitor and stem cells, i.e. sympathetic innervation functions as a protective factor for the development of the pathology.[146] The opposite occurs in epithelial neoplasms in which sympathetic innervation is increased leading to reactivation of developmental pathways.[147]

FETAL DEVELOPMENT OF THE PARASYMPATHETIC NERVOUS SYSTEM

The parasympathetic system consists of the oculomotor, facial, glossopharyngeal, and vagus nerves, several of which are crucial for the establishment of social interactions, especially in newborns, as they allow the detection of environmental stimuli, including the human voice, and the performance of behaviors such as head turning, smiling, sucking, and swallowing.[148]

The parasympathetic ganglia originate from NCCs at the cranial and sacral levels, specifically from the vagal NCCs, similarly to the sympathetic and sensory ganglia.[24,148] During neuralization the neural folds fuse to form the neural tube and the NCCs then undergo a transition from epithelial to mesenchymal, delaminating and migrating throughout the embryo.[24]

Unlike the sensory and sympathetic ganglia, the parasympathetic ganglia develop later and are located deeper in the body, near or within the target organs.[24] For parasympathetic ganglia to emerge, preganglionic nerves are required that serve as niches for the precursors of Schwann cells.[149,150] If these preganglionic parasympathetic nerves are eliminated or significantly decrease in size, the corresponding parasympathetic ganglia do not form.[149,150] These precursors then invade the target organ, proliferating and differentiating into the parasympathetic neurons of the ciliary, pterygopalatine, lingual, submandibular, and optic ganglia.[149,150]

At the final site, under the influence of BMPs, Schwann cell precursors activate the transcription factors Ascl1 and Phox2b, which will induce the parasympathetic properties of these neurons.[50,51,151] Schwann cell precursors give rise not only to neurons, but also to the glial cells of the parasympathetic ganglia.[149,150] All Schwann cell precursors express neuregulin 1 and its receptors ErbB2 and ErbB3 and therefore deletions in the neuregulin 1 ligand lead to a significant decrease in the number of parasympathetic neurons.[149,150] Unlike sympathetic neurons, parasympathetic neurons do not depend on nerve growth factor for their development.[65]

After the parasympathetic neurons mature they will innervate their respective targets, requiring some of the growth factors for their survival, namely GDNF and neurturin.[37]

All developing parasympathetic ganglia express Mash1, Phox2a and Phox2b, and some of them depend on the expression of Phox2a and Mash1 for normal development.[115] All types of peripheral autonomic neurons, including parasympathetic neurons, do not develop in the absence of Phox2b.[152]

The NCCs destined for the heart delaminate from neural folds at the level of the hindbrain, migrating across or lateral to the somites, unlike sympathetic NCCs that migrate only in the rostral part of the somites.[125] Parasympathetic innervation of the heart becomes functional earlier than sympathetic innervation.[125] GDNF and neurturin are essential for the development of parasympathetic innervation, being involved in both trophic support and innervation shaping.[125] In

mice null for neurturin and Gfrα2 functional parasympathetic disturbances occur at the level of the lacrimal and salivary glands.[152] Cardiac NCCs express the semaphorin 3C receptor called plexin A2. Absence of semaphorin 3C can cause several congenital cardiac pathologies, including interrupted aortic arch and persistent truncus arteriosus.[125]

Vagal neural crest cells

It is through the vagus nerve, which is one of the major afferent pathways of the PNS, that the brain receives information about the body's inflammatory milieu, which then leads to activating neuroimmune responses to reduce the perceived inflammation.[1,153]

Vagal NCCs arise adjacent to the somites, rostral to head NCCs, and caudal to trunk and sacrum NCCs.[154] These cells give rise to parasympathetic, sympathetic, and sensory ganglia, and also contribute to heart, thyroid/parathyroid, thymus, lung, and pancreas formation.[154,155] In the absence of vagal NCCs, the development of pancreatic neurons and glial cells will be altered and the thymus will lose its vasculature as well as its T-cell proliferation capacity.[154,155]

FETAL DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM

The enteric nervous system (ENS) is the largest component of the peripheral nervous system, consisting of 200-600 million neurons, being essential for digestion, transport, absorption and excretion of nutrients and fluids, and also has protective functions, identifying toxins and pathogens that may have been ingested.[24,156,157] When errors occur in the development of the ENS, the clinical manifestations are typically constipation, vomiting, abdominal pain and growth delays.[158] Enteric neurons must be able to actively relax smooth intestinal muscle, and so in cases of aganglionosis, the intestines are tonically contracted, leading to functional constipation.[158]

The ENS modulates the immune cells in the intestinal wall, which in turn influences the gut biome and the formation of cancer, abdominal pain, and pathologies such as Parkinson's Disease.[159,160]

Enteric neurons are distributed along the digestive tract organized into two concentric networks of ganglion plexuses - the Auerbach or myenteric plexus and the submucosal plexus.[156,157,161] The submucosal plexus or Meissner's plexus consists of a concentric arrangement of enteric ganglia in the layer of tissue adjacent to the luminal epithelium – the submucosa – which is absent in the esophagus.[157] The myenteric plexus or Auerbach's plexus, however, is located between the longitudinal and circular smooth muscle layers.[157] These network in conjunction

ensure a coordinated series of muscle contractions - peristalsis - responsible for the movement of the intestinal contents along the gastrointestinal tract.[156,161]

The signaling pathways sonic hedgedog and the BMP2 and BMP4 are associated with plexus patterning, while neurotrophin-3 and the BMP2 and BMP4 with neural differentiation.[157] Neuregulin 1 and Notch, in turn, are associated with gliogenesis, while semaphorin 3A and neuturin are associated with axonal growth.[157]

The ENS receives various extrinsic stimuli from postganglionic sympathetic nerves, vagus nerve and dorsal root ganglia, stimuli that serve to coordinate intestinal function and activity in order to maintain homeostasis in the body.[24] The vagal afferents transmit information to the brain regarding luminal metabolites, irritants, and chemosensory stimuli, while the vagal efferents, which are parasympathetic, regulate digestion.[24] The sympathetic input regulates fluid transport, causes vasodilation in the intestinal tract and regulates hormone secretion by the enteroendocrine cells.[162]

The ENS is derived from two different neural crest lineages, both originating at the junction of the neural plate and the adjacent ectoderm.[158,163,164]

In the ENS, cells derived from the enteric neural crest act as the enteric neural progenitor population.[157] The ENS-fated NCCs derive mainly of the vagal and, in a smaller portion, the sacral segment of the neural tube.[156,165,166] To form a functional ENS along, NCCs of vague and sacral origin have to go through several processes, including migration, survival, proliferation and differentiation (neuronal or glial).[157]

Upon entry into the intestine, the enteric neural crest-derived cells are in an undifferentiated state and as such express Sox10, RET, p75, Phox2b, Ednrb and Mash1.[157] Enteric neural crest-derived cells whose fate is the neuronal lineage undergo negative regulation of Sox10 and p75, maintain RET and Phox2b expression and express markers such as PGP9.5, neurofilament, neuronal β-tubulin class III, HuC and HuD.[157] In enteric neural crest-derived cells whose destination is the glial lineage, these maintain the expression of Sox10 and p75, negatively regulate RET and increase the expression initially of B-FABP and later of S100 and GFAP.[157]

The cells derived from the pre-enteric neural crest migrate rostrocaudally into the intestine and also undergo inward radial migration, in order to form the myenteric and submucosal plexus.[158,167] Netrins are one of the ligands involved in the radial migration of enteric neural crest-derived cells that occurs after initial colonization of the intestine.[158] As enteric neural crest-derived cells migrate, these will also proliferate and eventually differentiate into neurons and glia, condensing into ganglia to form the two layers of ganglia that form the ENS.[158]

Glial cells are crucial for neuroprotection, maturation, synapse formation, and neurotransmitter release.[168]

Although most neural crest stem cells are repelled from entering the anterior intestine, the vagal NCCs are not, and therefore migrate to the anterior intestine and colonize the entire developing gastrointestinal tract.[163,164] A second migration of NCCs to the posterior intestine occurs from the sacral region.[169,170] Cells derived from the vagal enteric neural crest migrate in an oral to anal direction, whereas cells derived from the sacral enteric neural crest migrate in the opposite direction.[157] First, enteric neural crest-derived cells migrate to the region of the posterior myenteric plexus, and it is only in this layer that the neuronal-fated progenitors differentiate into neurons.[157,171] Secondarily, enteric neural crest-derived glial-fated and bipotent cells migrate to the luminal region of the submucosal plexus, where they differentiate into glia and neurons, respectively.[157] In addition, glial cells can migrate to the mucosal layer to form units together with blood vessels, epithelial and immune cells.[171]

After neurogenesis, neuron-fated progenitors maintain the expression of RET and Phox2B, but also express enteric neuron-specific genes, namely Tubb3, Nf, TH, PGP9.5 and Elavl4.[157] As they differentiate they also become negative for glial markers and no further proliferation occurs.[157]

The other lineage that gives rise to enteric nerve system neurons are the precursors of Schwann cells, which colonize the axons of the vagus nerve as it innervates the esophagus and stomach.[149] If there is a decrease in the size of the vagus nerve, then the development of enteric nerve system neurons in the esophagus will be altered.[149] There is a second migration of precursor Schwann cells into the GI tract, which occurs after birth and accompanies the mesenteric and pelvic nerves.[172]

NCCs entering the GI tract from the rostral and caudal ends show behavioural differences between each other, with the former being much more invasive and having much higher levels of the RET receptor.[173] The RET receptor is expressed by enteric neural crest-derived cells as they migrate into the intestine and has four ligands, namely GDNF, neurturin, artemin, and persephin, which activate RET by binding to the coreceptors GFRα1, GFRα2, GFRα3, and GFRα4 receptors, respectively.[158] RET signaling is essential for the survival, proliferation and migration of NCCs into the intestine.[38,173,174] The RET coreceptor, GFRα1, and the GDNF ligand are, as mentioned above, the main activators of RET during fetal development, therefore, the deletion of RET/Gfrα1/GDNF results in the absence of enteric ganglia in the distal colon, a typical feature of Hirschsprung's Disease, which is a life-threatening developmental disorder.[174–181] Constitutively active mutations in RET have been causally associated with inherited cancer syndromes such as multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma.[158]

BMP2 and BMP4 and the transcription factor Ascl1 are crucial for migration. differentiation and maturation of enteric neurons.[182–184]

Similar to sympathetic neurons, ENS neurons go into apoptosis in the absence of signaling by the tyrosine kinase receptor ErbB3.[185]

Innervation of the gut by vagal axons depends on chemoattraction and chemorepulsion between the surrounding mesenchymal tissue and the developing enteric neurons.[186]

Endothelin3 and endothelin receptor type B are also essential for normal proliferation of enteric precursors.[187,188] The deletion or hypomorphic mutation of the endothelin receptor type B or endothelin 3 can cause Hirschsprung's Disease, usually in the context of Waardenburg syndrome type 4.[158] This disorder causes pigmentation defects, deafness, dysmorphic facial features, and aganglionic megacolon.[158] The deletion of endothelin 2 also leads to colorectal aganglionosis.[187,188]

After differentiation of enteric neurons and glia, they are also dependent on NT3-TrkC and its interactions with the BMP4 signaling pathway.[189]

Activation of the serotonin 5-HT4 receptor in animal models led to stimulation of ENS neurogenesis, and glial cells can be used to generate enteric neurons in response to neuronal injury, which may indicate a possible regenerative capacity of the ENS.[190–192]

Several transcription factors are important for the development of the ENS, with most of them influencing either the expression of RET - Sox10, Pax3 and Phox2B or endothelin receptor type B - Sox10.[51,193–195]

Heterozygous mutations in Sox10 can cause Waardenburg syndrome type 4 and Sox10-null mice undergo apoptosis even before their entry into the anterior intestine.[158,196] In addition, to maintain progenitor identity, enteric neural crest-derived cells need to maintain Sox10 expression in order to inhibit neural differentiation.[157]

Phox2b is a transcription factor required for RET expression in enteric neural crest-derived cells and heterozygous mutations of Phox2B may cause congenital central hypoventilation syndrome.[51]

Pax3 is required for ENS development and is also responsible for activating RET and Sox10.[193] Like other transcription factors the heterozygosity of Pax3 causes Waardenburg syndrome type 4 without Hirschsprung's disease.[193,197]

The sonic hedgehog ligands and Indian hedgehog proteins are expressed by the intestinal epithelium during the development of the ENS and are essential for concentric patterning of the intestinal wall.[198,199] Mutations in sonic hedgehog ligands result in an excessive number of enteric neurons and inadequate colonization of the villi by enteric neuron cell bodies.[198,199] Loss of indian hedgedog ligand, on the other hand, causes dilatation of intestinal segments and aganglionosis of parts of the gastrointestinal tract.[198]

Semaphorin 3A is expressed by the inner mesenchyme, but specifically affects sacral enteric neural crest-derived cells, while its coreceptor, neuropilin-1, is expressed in all enteric neural crest-derived cells.[158]

Finally, inadequate production of retinoic acid, for example due to vitamin A deficiency, can lead to defects in the development of the ENS.[200]

THE DIFFERENT COMPONENTS OF THE AUTONOMIC NERVOUS SYSTEM AND THEIR ROLE

The role of the sympathetic noradrenergic system

The noradrenergic sympathetic system is one of the structures responsible for regulating blood flow during various activities of daily living, such as standing, exercising, adapting to food intake, and regulating body temperature.[201,202]

The heart and coronary arteries are innervated by this system.[201] Sympathetic noradrenergic stimulation at this site results in increased strength and rate of cardiac contraction, as well as increased spontaneous depolarization.[201] The sympathetic noradrenergic nerves also innervate the smooth muscle cells of the arteriolar walls, which allows, by regulating the calibre of the arterioles, which determines the total peripheral resistance to blood flow, to also regulate blood pressure.[201] Finally, the sympathetic noradrenergic system is also responsible for regulating blood levels through various organs such as the kidneys, pancreas, thyroid, and salivary glands.[201]

Given the sites innervated by the sympathetic noradrenergic system, one would expect that lesions in this system would manifest clinically by dysregulation of the cardiovascular system.[155,201] Indeed, it does, and one of the manifestations of sympathetic noradrenergic injury is orthostatic hypotension, which is characterized by a 20 mmHg drop in systolic blood pressure or a 10 mmHg drop in diastolic blood pressure, 2 to 5 minutes after switching from sitting to standing.[201] Furthermore, communication failures between the noradrenergic sympathetic system and its target can lead to postprandial hypotension, inability to tolerate extreme temperatures, exercise intolerance, and, in the case of communication with the head, ptosis and miosis, as occurs in Horner's Syndrome.[24,201]

The role of the sympathetic cholinergic system

The sympathetic cholinergic system is primarily responsible for mediating sweating, and a failure in communication from this system to its target can lead to anhidrosis, a condition in which the sweat glands produce little or no sweat, a sign present in Horner's Syndrome along with

miosis and ptosis.[24,201] If noradrenergic sympathetic failure occurs, severe hypostatic hypotension will occur, as seen above, but sweating will occur normally, since the cholinergic sympathetic system remains intact.[131,201] As an example of a pathology in which there is failure of both the noradrenergic sympathetic system as well as the cholinergic sympathetic system, we have autoimmune autonomic ganglionopathy, in which the patient presents with orthostatic hypotension simultaneously with anhidrosis. [131,201]

The role of the sympathetic adrenergic system

The sympathetic adrenergic system is the main hormonal component of the ANS, since it is responsible for mediating the secretion of adrenaline.[201,203] Adrenaline, together with insulin and glucagon, is responsible for the regulation of blood glucose levels.[201] In case of failure of the sympathetic adrenergic system, changes in insulin secretion can occur, leading consequently to hypoglycemia.[143,201] However, this effect rarely manifests itself since the other effector systems generally remain intact.[201]

The role of the parasympathetic cholinergic system

The parasympathetic cholinergic system is responsible for digestion and calorie management, that is, whether calories are stored or eliminated.[201,203] Stimulation by this system leads to increased salivation and lacrimation, miosis, increased secretion of gastric acid, increased contraction of intestinal smooth muscle, increased bladder tone, and contributes to erection of the penis. [201] Parasympathetic cholinergic system failure therefore leads to clinical manifestations such as xerophthalmia and xerostomia, urinary retention and constipation.[201]

At the cardiac level, unlike the sympathetic noradrenergic system, the parasympathetic cholinergic system leads to decreased heart rate and decreased auriculoventricular electrical conduction.[125,201]

The main nerve of the parasympathetic cholinergic system is the vagus nerve, which is responsible for innervation of the heart muscle, most of the digestive system, and the splanchnic organs.[148,201] Since the vagus nerve also innervates the enteric system it is difficult to distinguish which pathologies are due to changes in the parasympathetic cholinergic system compared to the enteric system.[148,201]

AUTONOMIC REGULATION OF THE FETAL HEART

One of the key needs during fetal maturation is the development of the autonomic ability to adapt to varying levels of supply and demand in the organism.[204]

The heart is extensively innervated by autonomic nerves.[205] The parasympathetic components of the extrinsic cardiac nervous system originate from the vagus nerve.[205,206] The thoracic, cervicothoracic (stellate) and cervical ganglia mediate extrinsic sympathetic innervation.[207,208] It should be noted, however, that the vagus nerve also contains sympathetic fibers and that the sympathetic nerves also contain parasympathetic fibers.[206–208]

The autonomic tone is mainly regulated by baroreceptors, chemoreceptors and mechanical stress receptors that are located in the heart and in the great vessels.[205] There is also a network of ganglionic plexuses in the atria near the pulmonary vein ostia, formed by clusters of intrinsic ganglia, which attenuate interactions between the intrinsic and extrinsic nervous systems.[209] These ganglionic plexuses have both adrenergic and vagal fiber innervation.[209]

At the end of the second trimester, there is a first maturation of the parasympathetic branch, which can be seen in the differentiation of the lateral zone of the hypothalamus and in the increase in myelination of the vagus nerve.[204] From the 32nd week of pregnancy, the modulatory capacity of the vagus nerve develops.[204] This is evidenced both by baroreflex responsiveness and by the observation of an increase in respiratory sinus arrhythmia.[204] The development of sympathetic responsiveness may be observed in the coordination of fetal movements and heart rate acceleration.[204]

Sympathetic activation and vagal modulation both increase with fetal age, and the transition period between the second and third trimesters is characterized by physiological decelerations due to vagal regulation, followed by an increase in HRV.[204]

Role of heart rate variability

HRV, which consists of the variation in heart rate over time, is a non-invasive method that allows us to assess the functioning of the ANS and how it influences heart rate.[210] HRV is also a reflection of the balance between the parasympathetic and sympathetic nervous systems.[211]

Vagal activity is reflected in respiration-related short-term variations in the cardiac cycle, whereas modulation of sympathetic tone is reflected in long-term variations.[211]

Fetal heart rate in animal models

There is a decline in baseline fetal heart rate (FHR) with gestational age in the human fetus, a decline that occurs due to maturation of vagal parasympathetic functional control.[212,213] In the absence of autonomic activity, HRF appears to decrease progressively with gestational age and this may be due to an increase in muscle mass and cardiac work.[214]

The baseline FHR is regulated by a combination of intrinsic and extrinsic mechanisms, the latter being the sympathetic and parasympathetic branches of the ANS.[214]

In the high-voltage slow activity behavioral state the baseline HRF tends to be consistently higher compared to the low-voltage fast activity state.[214] This fact is explained by the increase in beta-sympathetic tone in the high-voltage slow activity behavioral state.[214] During the state of rapid low-voltage activity there is increased parasympathetic activity and decreased sympathetic activity resulting in decreased basal HRF.[214,215] Conversely, during the high-voltage slow-activity state sympathetic activity increases and parasympathetic activity decreases, leading to increased basal HRF.[214,215] Thus, each behavioral state has unique autonomic effects on FHR.[214] These changes in baseline HRF due to behavioral states are due solely to sympathetic activity however the mechanisms by which these fetal cardiovascular changes occur are still unknown.[216]

In the lamb fetus, parasympathetic cardiac function emerges at 60 days gestation, whereas adrenergic cardiovascular function emerges only from 85 days gestation.[217,218] The parasympathetic tone on resting heart rate in the fetal lamb was weak before 130 days gestation, in contrast to the sympathetic tone that was constant throughout gestation.[219] One study indicated that in the fetal lamb parasympathetic tone would be fully developed at 120 days gestation and sympathetic tone from 101 days.[217] However, although sympathetic tone finishes its development later, it becomes active earlier compared to parasympathetic tone.[218] Another study associated increased sympathetic and parasympathetic tone with the period from 85% gestation to term, meaning that in the last 15% of gestation the autonomic effects on FHR develop markedly.[214]

In animal models, there is a progressive increase in parasympathetic tone as term approaches and a decrease in sympathetic tone.[212]

Atrial fibrillation and autonomic nervous system abnormalities

Several cardiac consequences, including the development of atrial fibrillation, can result from autonomic dysfunction.[211,220] Sympathetic fibers promote the onset of arrhythmias by transiently increasing calcium synapses, since calcium entry into the presynaptic neuron is essential for neurotransmitter release.[221] Beta-adrenergic pathways increase calcium entry into the

presynaptic neuron and spontaneous calcium release from the sarcoplasmic reticulum increases.[221] This leads to neurotransmitter release and consequently increased action potential frequency.[221]

In contrast, by shortening action potential duration and refractory period, vagal stimulation contributes to the development of atrial fibrillation.[211] The neurotransmitter acetylcholine activates M2 muscarinic receptors, which modulate cardiac ion channels by activating the inwardly rectifying potassium channel (IKACh).[222,223] This channel is found in the sinoatrial node and atria and helps to regulate heart rate by accelerating repolarization and modulating cyclic (cAMP).[222,223]

Sympathetic and vagal innervation have different anatomical distributions of autonomic fibers.[224] Although sympathetic and parasympathetic terminals are both located close to the target cells and close to each other, they are not distributed in the same way.[224] The diminution of norepinephrine release by sympathetic nerves in response to acetylcholine release by neighboring vagal nerves is an example of the importance of this distribution and how stimulation of one system can affect the function of the other.[224]

The sympathetic nervous system and the parasympathetic nervous system differ not only in their distribution, as we have seen, but also in the latency period and the duration of the response.[224] The adrenergic response is slower, taking seconds to activate the target site, whereas the response to cholinergic stimulation occurs in milliseconds.[224]

In addition, the ganglionic plexuses play an important role in the innervation of the heart, and ablation of these plexuses can result in vagal denervation of the left atrium and the onset of atrial fibrillation.[211]

Changes in autonomic regulation in fetuses with congenital heart disease

Changes in perinatal autonomic function are associated with fetal exposure to alcohol, smoking, maternal depression and other stressors.[225]

Fetuses with congenital heart disease have a higher fetal heart rate and a lower level of HRV, differences that are detectable as early as 19 weeks of gestation.[225] This suggests that from the beginning of fetal gestation, the development of the autonomic nervous system may be altered in fetuses with congenital heart disease.[225] By detecting these changes early in pregnancy, fetal heart rate monitoring may be a valuable early risk assessment tool in populations at high risk of congenital heart disease.[225]

DISCUSSION

One of the most important factors in the regulation of various physiological processes is the ANS, which is divided into the sympathetic and parasympathetic nervous systems.[203]

Neural crest cells, as we have seen, give rise to all components of the autonomic nervous system.[25,163,226] These cells then undergo a process of migrating and differentiating, which varies depending on the type of cell they will become and the organ targeted.[154,158,165] A number of transcription factors, proteins, channels and molecules are required for this process to take place, and if they're missing, for example due to a mutation, the autonomic nervous system will not develop normally.[45,51] There is still a lack of understanding of the full molecular and cellular mechanisms involved in neuronal survival, axon growth and target innervation of the autonomic nervous system.[2,4] If we are able to understand in detail how these mechanisms work, we may in the future be able to develop specific therapies to prevent the development of these pathologies, or at least to prevent the occurrence of serious consequences.

The development and regulation of the autonomic nervous system can be affected by exposure to factors such as stress, malnutrition and toxins during pregnancy.[10] Several pathologies, including cardiac arrhythmias, hypertension, diabetes mellitus and sudden fetal death syndrome, can result from a failure of the autonomic nervous system.[144,211,224] It is, therefore, not surprising that more and more studies are being conducted on this topic, given the significant contribution of the autonomic nervous system, especially the sympathetic nervous system, in various diseases.

HRV consists on the variation in the heart's beat-to-beat intervals and is one of the ways to assess the activity of the autonomic nervous system.[14,210] There is good evidence that FHR and HRV are reduced and increased, respectively, during pregnancy.[210,216] It is therefore important to study how this method can be used as a screening test for the prediction of lethal fetal complications associated with the dysregulation of the autonomic nervous system (Fig. (2)).

CONCLUSION

The development of the ANS is a complex and intricate process, and for this to occur normally, the synergistic and simultaneous functioning of each of the fundamental components is required.[152] Several factors can influence the development of the ANS and affect its regulation, which can lead to serious pathologies or even death.[10,145] In conclusion, the assessment of HRV can provide relevant information regarding the ANS, and perhaps in the future allow the prediction of the emergence of these same pathologies.[210]

APPENDICES

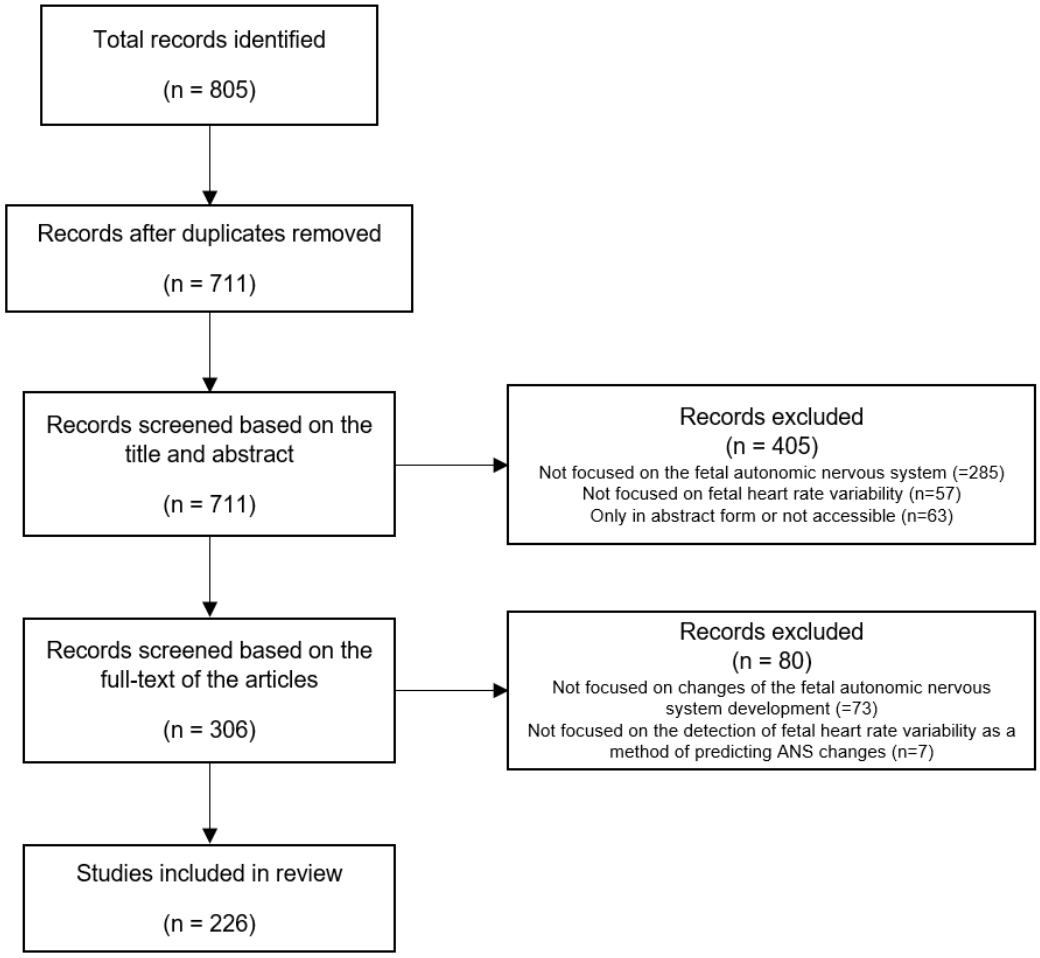

Fig. (1) – Methods flowchart

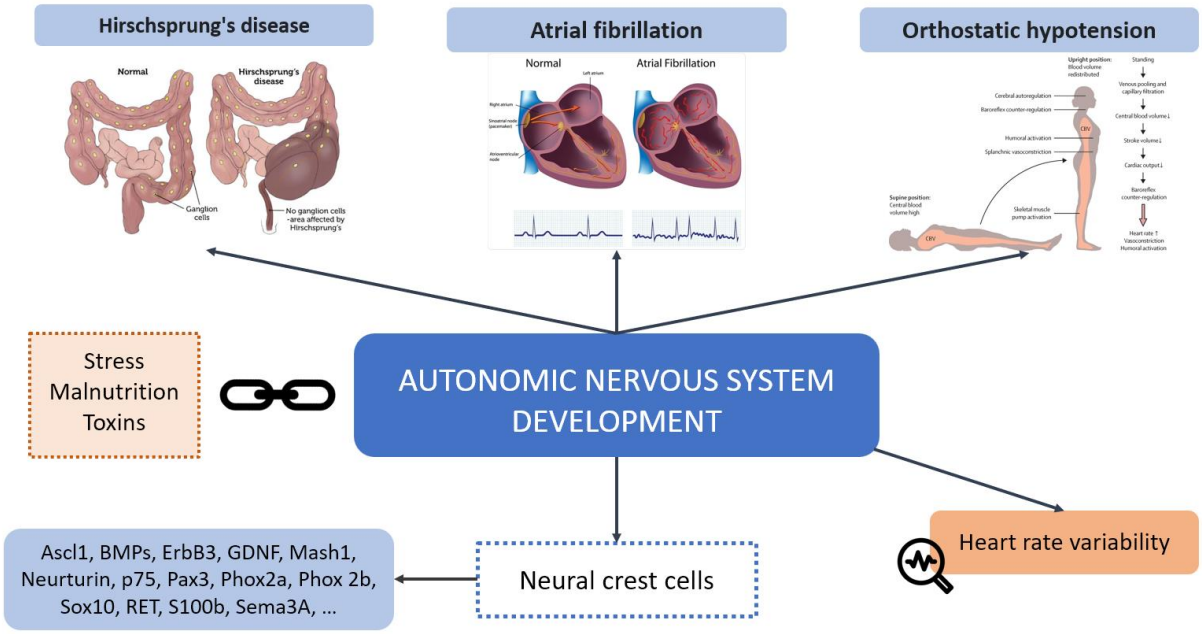

Fig. (2) – Graphical Abstract

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