

IN SITU HYBRIDIZATION STUDY OF THE DISTRIBUTION OF CHOLINE ACETYLTRANSFERASE mRNA AND ITS SPLICE VARIANTS IN THE MOUSE BRAIN AND SPINAL CORD

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Abstract—Choline acetyltransferase is the enzyme that catalyzes the biosynthesis of the neurotransmitter acetylcholine. Seven types of mRNA for choline acetyltransferase that differ in the 5′-noncoding region are transcribed from the cholinergic gene locus from different promoter regions and produced by alternative splicing in the mouse. Digoxigenin-labeled riboprobes and *in situ* hybridization histochemistry were used to investigate the expression of N1, R1, R2, R3, R4 and total choline acetyltransferase mRNA in the mouse CNS. The relative levels of choline acetyltransferase transcripts differed dramatically in distinct subdivisions of the mature cholinergic nervous system. Neurons hybridizing with antisense riboprobes for all of the five investigated splice variants (R1, R2, R3, R4 and N1) as well as those hybridizing with riboprobe for the common protein-coding region were found in a number of expected regions in the CNS. They include the basal forebrain, striatum, pontomesencephalic tegmentum, motor and autonomic nuclei of the brainstem, and spinal cord. Neurons with a moderate to very high level of expression of R1 and R2 splice variants were distributed in both the forebrain and brainstem nuclei. On the other hand, R3, R4 and N1 splice variants revealed a moderate to high level of expression in the brainstem motor and autonomic nuclei and ventral and lateral horns of the spinal cord compared to a low expression level in forebrain cholinergic structures. No expression of the N1, R1, R2, R3 and R4 splice variants was detectable in the neurons of the cerebral cortex, hippocampus and medial habenular nucleus. With the riboprobe for the common protein-coding region, the neurons of the medial habenular nucleus could be labeled at high level, while intrinsic cortical neurons were labeled at low level. Hippocampus revealed no significant hybridization for total choline acetyltransferase mRNA. These findings strongly suggested that: (1) R1 and R2 were the major splice variants expressed in the neurons of forebrain nuclei; (2) R1, R2, R3, R4 and N1 splice variants were almost equally expressed in the brainstem motor and autonomic nuclei and ventral and lateral horns of the spinal cord; (3) inferring from a paucity of other isoforms, M type choline acetyltransferase mRNA is a splice variant predominantly expressed in the cerebral cortex and medial habenular nucleus. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

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Abbreviations: ChAT, choline acetyltransferase; DEPC, diethyl pyrocarbonate; DIG, digoxigenin; PB, phosphate buffer; pChAT, peripheral type of choline acetyltransferase; PPTg, pedunculopontine tegmental nucleus; RT-PCR, reverse transcription–polymerase chain reaction; VACHT, vesicular acetylcholine transporter.

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Key words: cholinergic neuron, cholinergic gene locus, choline acetyltransferase gene organization, 5′-noncoding region, noncoding choline acetyltransferase exons, medial habenular nucleus.

Choline acetyltransferase (acetyl-CoA, choline O-acetyltransferase, ChAT; EC 2.3.1.6) is the enzyme that catalyzes the biosynthesis of the neurotransmitter acetylcholine from acetyl-coenzyme A and choline in the cytoplasm, which is subsequently translocated into synaptic vesicles via the vesicular acetylcholine transporter (VACHT). Acetylcholine is a major neurotransmitter that plays a pivotal role in such fundamental brain processes as learning, memory, arousal, sleep and movement (Woolf, 1991; Butcher and Woolf, 2004). In Alzheimer disease, subnormal level of expression of ChAT mRNA per cholinergic cell was demonstrated (Strada et al., 1992) and selective loss of cholinergic neurons has been observed in the basal forebrain and especially in the nucleus basalis magnocellularis (Meynert) (Kása et al., 1997; Härtig et al., 2002; Schliebs and Arendt, 2006). The cholinergic neurons are affected also in other neurodegenerative disorders such as Huntington disease (Ferrante et al., 1987) and amyotrophic lateral sclerosis (Kato, 1989; Virgo et al., 1992); therefore, morphologic studies together with genetic and molecular analysis of ChAT will provide the possibility to understand the physiologic and pathologic states of cholinergic neurons. The expression of common and peripheral (pChAT) types of ChAT mRNA is found in a variety of non-neuronal tissues and organs. pChAT is generated from a splice variant of ChAT mRNA that lacks coding exons from 6 to 9 (Tooyama and Kimura, 2000; Bellier and Kimura, 2007). In particular, the human and rat placenta (Oda et al., 1996, 2004; Pfeil et al., 2004) and human and murine immune cells (Ogawa et al., 2003; Kawashima et al., 2007) are well known to contain large amounts of acetylcholine and its synthesizing enzyme ChAT.

Regulation of ChAT production and activity under physiological and pathological conditions is important for the development and neuronal activities of cholinergic systems. ChAT regulation is likely to involve the spatial and temporal control of transcription, as well as regulation at the level of RNA processing, transport, turnover or translation (Wu and Hersh, 1994; Eiden, 1998). The enzyme ChAT is encoded by a single gene in all species studied so far (Wu and Hersh, 1994). Three noncoding exons (R, N and M) have been identified upstream of the first common protein coding exon and at least three separate promoters



GPR155: Gene organization, multiple mRNA splice variants and expression in mouse central nervous system

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ABSTRACT

Emerging evidence suggests that GPR155, an integral membrane protein related to G-protein coupled receptors, has specific roles in Huntington disease and autism spectrum disorders. This study reports the structural organization of mouse GPR155 gene and the generation of five variants (*Variants 1–5*) of GPR155 mRNA, including so far unknown four variants. Further, it presents the level of expression of GPR155 mRNA in different mouse tissues. The mRNAs for GPR155 are widely expressed in adult mouse tissues and during development. In situ hybridization was used to determine the distribution of GPR155 in mouse brain. The GPR155 mRNAs are widely distributed in forebrain regions and have more restricted distribution in the midbrain and hindbrain regions. The highest level of expression was in the lateral part of striatum and hippocampus. The expression pattern of GPR155 mRNAs in mouse striatum was very similar to that of cannabinoid receptor type 1. The predicted protein secondary structure indicated that GPR155 is a 17-TM protein, and *Variant 1* and *Variant 5* proteins have an intracellular, conserved DEP domain near the C-terminal.

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1. Introduction

An integral membrane protein termed GPR155 has been identified by systematic search for homologies to known G-protein coupled receptors (GPCRs). This protein appears to have specific roles in the brain based on the reports, characterizing its significant dysregulation in the caudate nucleus of Huntington disease patients and animal models [1,2] and in lymphoblastoid cells of humans with autism spectrum disorders [3]. At present, however, the gene organization, tissue distribution and localization of GPR155 in central nervous system have been elucidated at a less extent.

We report the structural organization of mouse GPR155 gene by sequencing all intron–exon boundaries and the generation of five variants of GPR155 mRNA including so far unknown four variants in the mouse (GenBank No. HM161763; HM161764; HM161765; HM161766). The sequence prediction shows that, unlike typical GPCRs, GPR155 is a 17-TM protein. *Variant 1* and *Variant 5* proteins have an intracellular, conserved DEP domain near the C-terminal. Further, this study presents the expression levels of GPR155 mRNA in different mouse tissues. Localization of the mRNA for GPR155 was performed by in situ hybridization, which reveals clues regarding the role of this receptor in motor, cognitive and limbic systems.

2. Materials and methods

2.1. In silico cloning and analysis of genomic structure of GPR155

The mouse DNA sequence from clone RP23-463K8 on chromosome 2 (GenBank No. AL954713), comprising several genes including the gene for GPR155, was analyzed with GENSCAN (<http://genes.mit.edu/GENSCAN.html>). The predicted peptide and coding sequences were set to query, by means of *tblastn* and *tblastx*, against *nucleotide collection (nr/nt)* database by BLAST search site (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Except for the reference mRNA and protein sequences (GenBank No. NM_001080707; NP_001074176), high homology was found with a protein sequence CAM25138. This sequence was used to obtain a protein-coding sequence, which was then subjected to *nucleotide blast* by BLAST search site. Multiple alignment, homology search and ORF prediction were performed by GENETYX 8.0 and SIM4 (<http://pbil.univ-lyon1.fr/members/duret/cours/inserm210604/exercise4/sim4.html>) for aligning cDNA and genomic DNA to identify exon and intron regions.

Multiple alignment of amino acid sequences predicted from GPR155 splice variants and analysis of interspecies homologies were performed with CLUSTALW2 (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>). Analysis for signal peptide was performed by Signal IP 3.0 (<http://www.cbs.dtu.dk/services/SignalP/>). PROSITE (<http://au.expasy.org/prosite/>) and InterProScan (<http://www.ebi.ac.uk/Tools/InterProScan/>)

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Histological Determination of the Areas Enriched in Cholinergic Terminals and m2 and m3 Muscarinic Receptors in the Mouse Central Auditory System

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ABSTRACT

Cholinergic projections to auditory system are vital for coupling arousal with sound processing. Systematic search with *in situ* hybridization and immunohistochemistry indicated that the ventral nucleus of the medial geniculate body and the nucleus of the brachium of the inferior colliculus constituted cholinergic synaptic sites in the brainstem auditory system, containing a significant number of cholinergic axon terminals and m2 receptor-expressing cell bodies. Anat Rec, 293:1393–1399, 2010. © 2010 Wiley-Liss, Inc.

Key words: central auditory system; muscarinic receptors; vesicular acetylcholine transporter

Cholinergic neuronal connections in the central auditory system have been known to play essential roles not only in basic neuromodulatory processes (Habbicht and Vater, 1996; Yigit et al., 2003) but also in behavioral arousal or attention (Metherate and Ashe, 1993; Hsieh et al., 2000), plastic response (Ji et al., 2001, 2005; Ji and Suga, 2008), and cognitive functions. In Alzheimer disease, marked degenerative changes are frequently found throughout major sites of the ascending auditory pathway including the primary auditory cortex, the ventral nucleus of the medial geniculate body, and the central nucleus of the inferior colliculus (Ohm and Braak, 1989; Sinha et al., 1993).

At the earlier stages of Alzheimer disease, O'Mahony et al. (1994) found a significant dysfunction in the midlatency response, indicating an impairment of the ascending cholinergic system that originates in the pedunculopontine tegmental nucleus and mostly terminates in the thalamus. Buchwald et al. (1991) also observed a close relation between the auditory and ascending cholinergic systems by pharmacological manipulation with a cholinergic agonist and antagonist.

Morphologic evaluation of the cholinergic fibers and receptors responsible for the function of the central auditory system has been little accomplished except for the

cochlear nucleus, where the distribution density of cholinergic fiber–receptor systems has been reported to be regionally variable (Frostholt and Rotter, 1986; Chen et al., 1995; Yao and Godfrey, 1995, 1999; Yao et al., 1996; Jin et al., 2005; Jin and Godfrey, 2006). Tsutsumi et al. (2007) studied the distribution of cholinergic components in the precerebellar nuclei by using vesicular acetylcholine transporter (VACHT) immunohistochemistry and provided evidence that mesopontine cholinergic neurons negatively regulate neocortico-ponto-cerebellar projections at the level of pontine nuclei. Because VACHT is synthesized in major cholinergic neurons and localized

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Lineage-Specific Purification of Neural Stem/Progenitor Cells From Differentiated Mouse Induced Pluripotent Stem Cells

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Key Words. Induced pluripotent stem cells • Neural stem cell • Neural differentiation • Stem/progenitor cell

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ABSTRACT

Since induced pluripotent stem (iPS) cells have differentiation potential into all three germ layer-derived tissues, efficient purification of target cells is required in many fields of iPS research. One useful strategy is isolation of desired cells from differentiated iPS cells by lineage-specific expression of a drug-resistance gene, followed by drug selection. With this strategy, we purified neural stem/progenitor cells (NSCs), a good candidate source for regenerative therapy, from differentiated mouse iPS cells. We constructed a bicistronic expression vector simultaneously expressing blasticidin S resistance gene and DsRed under the control of tandem enhancer of a 257-base pair region of nestin second intron, an NSC-specific enhancer. This construct was efficiently inserted into the iPS genome by *piggyBac* transposon-mediated gene transfer, and the established subclone was differentiated into NSCs in the presence or absence of blasticidin S. Consequently, incubation with blasticidin S led to purification of NSCs from differentiated iPS cells. Our results suggest that a lineage-specific drug selection strategy is useful for purification of NSCs from differentiated iPS cells and that this strategy can be applied for the purification of other cell types. STEM CELLS TRANSLATIONAL MEDICINE 2013;2:420–433

INTRODUCTION

In 2006, Yamanaka's group first reported a method for reprogramming mouse fibroblasts to pluripotent stem cells, so-called induced pluripotent stem (iPS) cells, by the introduction of four transcription factors [1]. Subsequently, many groups succeeded in reprogramming various cell types using various reprogramming strategies [2–6]. Since patient-specific iPS cells could overcome problems such as ethical issues and immunological rejection, iPS cells are predicted to become a powerful tool for cell replacement therapy. Furthermore, establishment of disease-specific iPS cells promises to become an invaluable tool for disease investigation and drug development [7]. However, undifferentiated iPS cells caused teratoma formation when they were transplanted into animals, and disease-specific iPS cells should be differentiated into target cells that are the etiology of disease. Therefore, isolation of a single cell type from iPS cells would be an important step in many lines of research. Although enormous efforts have been devoted to developing effective differentiation methods that yield highly purified cell populations of target cells, it is difficult to obtain a single cell type from iPS or embryonic stem (ES) cells. One possible solution is isolation of target cells by fluores-

cence-activated cell sorting (FACS) with a specific antibody. However, this technique can only be applied to the cells on which lineage-specific cell surface antigen is expressed. Another possible solution is the expression of fluorescence proteins under the control of lineage-specific promoter or enhancer in target cells and isolation of desired cell population using FACS [8–10]. As an easier method, several reports have indicated that lineage-specific expression of a drug-resistance gene and subsequent drug selection strategy were useful for the selection of neural precursors [11], cardiomyocytes [12], insulin-producing cells [13], and endothelial cells [14] from differentiated ES cells.

It is widely accepted that the adult central nervous system does not regenerate spontaneously. Since neural stem/progenitor cells (NSCs) are capable of self-renewal and have differentiation potential into various types of cells including neurons, astrocytes, and oligodendrocytes, they are recognized as candidate sources for neurodegenerative disorders. Although several molecules, such as nestin [15], Sox1 [16], and musashi1 [17, 18] are known as markers of NSCs, antibodies against these markers cannot be used to isolate NSCs by FACS because these markers are not cell surface antigens. Lineage-specific

Overview and assessment of the histochemical methods and reagents for the detection of β -galactosidase activity in transgenic animals

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Abstract Bacterial β -galactosidase is one of the most widely used reporter genes in experiments involving transgenic and knockout animals. In this review we discuss the current histochemical methods and available reagents to detect β -galactosidase activity. Different substrates are available, but the most commonly used is X-gal in combination with potassium ferri- and ferro-cyanide. The reaction produces a characteristic blue precipitate in the cells expressing β -galactosidase, and despite its efficiency in staining whole embryos, its detection on thin tissue sections is difficult. Salmon-gal is another substrate, which in combination with ferric and ferrous ions gives a reddish-pink precipitate. Its sensitivity for staining tissue sections is similar to that of X-gal. Combining X-gal or Salmon-gal with tetrazolium salts provides a faster and more sensitive reaction than traditional β -galactosidase histochemistry. Here, we compare the traditional β -galactosidase assay and the combination of X-gal or Salmon-gal with three tetrazolium salts: nitroblue tetrazolium, tetranitroblue tetrazolium and iodonitrotetrazolium. Based on an assessment of the sensitivity and specificity of the different combinations of substrates, we are proposing an optimized and enhanced method for β -galactosidase detection in histological sections of the transgenic mouse brain. Optimal staining was obtained with X-gal in combination with nitroblue tetrazolium, which provides a faster and more specific staining than the traditional X-gal combination with potassium ferri- and ferro-cyanide. We recommend the X-gal/nitroblue tetrazolium staining mixture

as the first choice for the detection of β -galactosidase activity on histological sections. When faster results are needed, Salmon-gal/nitroblue tetrazolium should be considered as an alternative, while maintaining acceptable levels of noise.

Keywords β -galactosidase · *lacZ* · Nitroblue tetrazolium · Salmon-gal · X-gal

lacZ as a reporter gene in molecular biology

Reporter genes are used in myriad transgenic experiments, from *Drosophila* to mammals, during embryonic development and in the postnatal period. One of the most commonly used reporter genes in molecular biology is the β -galactosidase gene (*lacZ*) of *Escherichia coli* (Beckwith 1980; Cui et al. 1994; Takahashi et al. 2000; Burn 2012). In the mouse, *lacZ* has been used to identify the *cis*-acting DNA elements important for the regulation of gene expression, by knocking it into the endogenous gene or locus of interest or by randomly integrating into the genome a plasmid containing *lacZ* under the control of the regulatory elements of interest (Goring et al. 1987; Bonnerot and Nicolas 1993; Sekerková et al. 1997). The *lacZ* gene is frequently used in the detection of genetic recombination events by Cre-loxP and the detection of embryonic stem cell derivatives in cell fate mapping experiments (Petit et al. 2005; Joyner and Zervas 2006; Watson et al. 2008). The use of Cre recombinase allows tissue-specific and/or temporal control of *lacZ* expression. Cre-expressing mice are often used in combination with the Rosa26R mouse line, which has a ubiquitously expressed Cre-inducible *lacZ* (Soriano 1999).

The complete sequencing of human and mouse genomes led to the next important goal, functional analysis of more than 20,000 protein-coding genes. To help accomplish this

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MORPHOLOGICAL CHANGES IN THE PROPRIOCEPTORS OF HUMAN ANTERIOR CRUCIATE LIGAMENT DURING DEGENERATIVE OSTEOARTHRITIS

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ABSTRACT

Introduction/Aim: The anterior cruciate ligament (ACL) of the knee joint is one of the most investigated structures of the musculoskeletal system. Numerous studies are dedicated to the macro- and micro anatomy of human and animal ACL, but not many are focused on the proprioceptors of the ligament. The aim of this study was to establish a relationship between the proprioceptors and the stage of degenerative changes in the ACL.

Materials and Methods: Sixty ACL were harvested from patients admitted for knee replacement surgery. The samples were processed, histochemically and immunohistochemically stained according to standard laboratory protocols. Results were analysed and interpreted. The research was approved by the Ethics Committee of Medical University Pleven.

Results: Fifty-seven patients were included in our study – 37 females and 20 males. The average age of patients was 66.48 years. Degeneration of ACL was classified in four stages, and four different patterns of degeneration were observed: 1) necrosis and inflammation; 2) myxoid and cystic formation; 3) vascularisation with myxoid changes; 4) inflammation and hyalinosis. With the increasing severity of degeneration, the number of specialized nervous structures diminish.

Discussion: Our findings support the results from studies on animal models, conducted by other authors. Cabuk et al. reported a diminishing number of proprioceptors in the PCL of an osteoarthritic human knee. The lower number of proprioceptors could result in the onset or progression of the arthritic process.

Conclusion: Osteoarthritis involves all elements of the knee joint. Altered proprioception could be the reason for unsatisfied patients with artificial knees.

Keywords: ACL, proprioceptors, osteoarthritis,

INTRODUCTION/AIM:

The anterior cruciate ligament (ACL) of the knee joint is one of the most investigated structures of the musculoskeletal system, that continues to provoke debate between scientists and surgeons. It is thick, stout, intra-articular, but an extra synovial structure which acts to control rotational movement and anterior translation of the femur upon the tibia. ACL originates from a broad anterior intercondylar area of the tibia, passes from anterior to posterior, and inserts into the most posterior part of the medial aspect of the lateral femoral condyle. It is approximately 35mm long and 11mm in diameter on average. It receives its blood supply from the middle genicular artery and is exclusively innervated by the tibial nerve [1-3].

Numerous studies about macro- and micro anatomy of human or animal ACL can be found in the literature, but not many focused-on proprioceptors exist. Proprioceptors are specialized structures that detect changes in tension, acceleration, the direction of movement and the position of the knee joint. In one of the earliest, detailed light microscopic study in 1967, Freeman and Wyke [4] proposed classificational system of mechanoreceptors, that was modified by Hagert [5] in 2008:

- Type I (Ruffini corpuscle) nerve endings are slow to adapt and have low mechanical thresholds that can signal a static joint's position, the intra-articular pressure and the amplitude and velocity of a movement (Fig. 1).

- Type II (Pacini corpuscle) nerve endings are low-threshold and fast-adapting nerve endings that are responsible for signalling the acceleration and deceleration of joint rotation (Fig. 2).

- Type III (Golgi-Mazzoni corpuscle) nerve endings are low-threshold and slow-adapting nerve endings that can measure the tension of ligaments when the joint is at the extremes of its movement range (Fig. 3).

- Type IV (free) nerve endings are high-threshold



ACETYLCHOLINE AND ITS SYNTHESIZING ENZYME CHOLINE ACETYLTRANSFERASE IN THE ENTERIC NERVOUS SYSTEM

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ABSTRACT

The enteric nervous system is the largest and most complex division of the peripheral nervous system. Located in the wall of the gastrointestinal tract, it is a regulatory and coordination unit of the nervous system. Neurochemical, pharmacological, and functional studies describe three main classes of neurons in the enteric nervous system— primary afferent, interneurons and motor neurons. These are grouped in ganglia, which are connected and form plexuses. Acetylcholine is a major neurotransmitter that plays a pivotal role in the enteric nervous system and several non-neuronal structures. Internal cholinergic neurons and vagus terminals in the enteric nervous system use acetylcholine as the main excitatory neurotransmitter, regulating motility and mucosal function in the digestive system. The enzyme choline acetyltransferase (ChAT) that catalyzes the synthesis of acetylcholine represents the most specific cholinergic marker. Recent markers used to visualize cholinergic structures are the splicing variants of ChAT mRNA that are transcribed from the ChAT gene. Different alternatively spliced ChAT mRNA variants are transcribed in many animal species, including humans. In the mouse, seven variants in the 5'-noncoding region and two variants that differ in their coding region are described. Morphological, genetic and molecular analysis of ChAT and its splicing variants, as the most reliable and frequently used marker for cholinergic structures, would contribute to a better understanding of the physiological and pathological states of cholinergic neurons in the enteric nervous system.

Keywords: enteric nervous system, acetylcholine, choline acetyltransferase, mRNA splicing variant, mouse,

BACKGROUND

A historical perspective on the enteric nervous system

The study of the enteric nervous system (ENS) dates back to the 19th century when German anatomists Leopold Auerbach and Georg Meissner discovered the existence of neurons in the intestinal wall – myenteric and submucosal plexuses. In 1899, two English scientists – Bayliss and Starling isolated a loop of the dog intestine

and performed experiments on the function of neurons inside the intestinal wall. They described the “The low of the intestine”. This was the first demonstration of the peristaltic reflex and the ability of the enteric nervous system to function independently of the central nervous system. Silver impregnation methods were employed by R. Y. Cajal to establish that the intestinal neurons are of many different types. The first attempts for morphological classification were made by A. S. Dogiel, who classified the enteric neurons according to their morphological features [1]. In the 20th century, multiple experiments and further classification were made to sustain or refute the discoveries of previous scientists.

Organization of the enteric nervous system, a mini-brain in the gastrointestinal tract

The enteric nervous system, as part of the autonomic nervous system, controls motor functions, local blood flow, mucosal transport and secretion and modulates the immune and endocrine functions of the gastrointestinal tract. The enteric nervous system has two major ganglionated plexuses: the myenteric (Auerbach plexus), which lies between the longitudinal and circular muscle layer and the submucosal (Meissner plexus), which is positioned in the submucosa. In large mammals, including humans [2], the submucous plexus is subdivided into the inner submucous (Meissner plexus), the outer submucous plexus (Schabadach or Henle plexus), directly adjacent to the circular muscle layer and an intermediate plexus located between the two. Non-ganglionated plexuses also supply the layers of the gastrointestinal tract [3, 4].

Types of enteric neurons

The neural apparatus of ENS is composed of many enteric neurons that can be classified according to their shape, projections, connections, functions, and neurochemistry [5, 6]. Different methods have been used to describe the functional classes of enteric neurons, including light and electron microscopy, immunohistochemistry, electrophysiological analysis, intracellular dyes, and retrograde tracing of neuronal projections [7, 8, 9]. Ac-

***De novo* design of a PCR-RFLP assay for detecting the leptin receptors single nucleotide polymorphism in hypothalamic nuclei**

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Abstract

Obesity in adulthood is associated with many comorbidities, such as cardiovascular diseases, type 2 diabetes, and cancers. The central melanocortin system is very important for the regulation of energy homeostasis and controlling body weight. The hypothalamic leptin-melanocortin regulation pathway involves signaling interactions between leptin (LEP), leptin receptors (LEPR), and melanocortin receptors (MC4R). The rs1137101 polymorphism of the LEPR gene is one of the polymorphisms with the highest allele frequency and is associated with increased body weight and high LEP levels due to impaired LEPR intracellular signaling. The aim of the current study is to design specific primers that amplify the region of LEPR containing rs1137101. DNA was isolated from peripheral blood leucocytes. The primers used for gradient PCR were *de novo* designed by Primer 3. The resulting 346 bp PCR fragment of the LEPR gene was visualized on a 2.5% agarose gel. The expected band size of 346 bp was detected only within the range between 45 °C to 58.6 °C. Within the tested temperature range, 53.8 °C and 56.2 °C were the optimal temperatures that showed lack of non-specific amplification, so 55 °C was chosen as appropriate annealing temperature for the tested primer pair.

Keywords: single nucleotide polymorphism, leptin receptors, obesity, gradient PCR, hypothalamic leptin-melanocortin regulation pathway.

Introduction

The high prevalence of obesity and type 2 diabetes is a leading public health problem of the 21st century. The body mass index (BMI) of children and adolescents has increased globally over the past decades [1]. Overweight children and adolescents have an increased risk of becoming overweight adults [2]. Obesity in adulthood is associated with several comorbidities, including but not limited to cardiovascular diseases, type 2 diabetes, and different forms of cancer [3, 4]. Various genetic, metabolic, behavioral, and environmental factors play a key role in the development of obesity [5].

Changes in BMI are related to energy balance, which is maintained via a dedicated homeostatic system. Regulation of energy homeostasis and control of body weight are mediated by the melanocortin system [6]. The hypothalamic leptin-melanocortin regulation pathway involves signaling interactions between leptin (LEP), leptin receptors (LEPR), and melanocortin receptors (MC4R) [7]. LEP is a peptide hormone secreted by unilocular adipocytes. It binds to LEPR expressed on neurons in several hypothalamic nuclei such as nucleus arcuatus and nucleus ventromedialis. Hormone-receptor binding stimulates post-translational cleavage of pro-opiomelanocortin (POMC) to melanocyte stimulating hormone, and further signaling through MC4R [6, 8]. Scientific data regarding the relationship between polymorphisms in LEP and LEPR genes and human obesity are still controversial. The rs1137101 polymorphism of the LEPR gene is one of the polymorphisms with the highest allele frequency and it is associated with increased body weight and high LEP levels due to impaired LEPR intracellular signaling [9, 10].

The aim of the current study was to design a new in-house assay for LEPR rs1137101 genotyping by PCR-RFLP. The goal of the experiment was to amplify a 346 bp fragment of interest from the LEPR gene.

Materials and methods

Genomic DNA was isolated from peripheral blood leucocytes using column-based DNA extraction (QIAmp DNA Blood Mini Kit, QIAGEN, USA). DNA was quantified using NanoDropTM One (ThermoScientific,

RESEARCH

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Gut colonization with vancomycin-resistant *enterococci* among patients with hematologic malignancies

Preslava Mihaylova Hristova^{1*}, Vladislav Milkov Nankov², Ivaylo Georgiev Hristov³, Stefan Venelinov Trifonov², Alexandra Sashova Alexandrova⁴ and Hristina Yotova Hitkova¹

Abstract

Background Vancomycin-resistant enterococci (VRE) are well known agents that colonize the gastrointestinal tract of immunocompromised patients, especially those with hematologic malignancies. The aim of the current study was to determine the incidence of and risk factors for colonization with VRE among patients with hematologic malignancies.

Materials For a nine-month period, all patients admitted to the Hematology ward at University Hospital in Pleven, Bulgaria who had hematologic malignancy and duration of hospitalization of more than 48 h were screened for colonization with VRE. The data collected from patients and their medical records during the entire hospital stay included: demographic characteristics, clinical information and information about all antimicrobials used. A longitudinal study was used to assess the risk factors and statistical analysis was performed using SPSS version 27.0.

Results A total of 119 patients were enrolled in the study. Colonization with VRE was established in 18 of them. One patient carried two species, resulting in a total of 19 VRE: 12 *Enterococcus gallinarum*, 4 *Enterococcus casseliflavus*, 2 *Enterococcus faecium* and 1 *Enterococcus faecalis*. VanA phenotype, with high-level resistance of vancomycin (MIC ≥ 256 $\mu\text{g/ml}$) and teicoplanin (MIC = 96 $\mu\text{g/ml}$), was demonstrated by one *E. faecium*, which carried *vanA*. The other *E. faecium* and *E. faecalis* expressed low-level resistance to vancomycin (MICs: 8 $\mu\text{g/ml}$ and 12 $\mu\text{g/ml}$), susceptibility to teicoplanin (MICs = 0.5 $\mu\text{g/ml}$) and *vanB* was detected. All *E. gallinarum* and *E. casseliflavus* showed low-level resistance to vancomycin and susceptibility to teicoplanin. *E. gallinarum* strains were positive for *vanC1* and *E. casseliflavus* for *vanC2*. Only two patients were colonized with *vanA* or *vanB* enterococci and the rest 16 were positive for *vanC*. The univariate analysis revealed that patient's age (70–79 years; $p = 0.025$) and multiple myeloma ($p = 0.001$) are risk factors for VRE acquisition among the investigated patients. In addition, the multivariate analysis confirmed that patient's age (70–79 years) is an independent risk factor for VRE colonization.

Conclusions Our results showed that 15.1% of patients with hematologic malignancies were colonized with VRE. There was a distinct prevalence of *vanC* enterococci. Among the analyzed risk factors, advanced age and multiple myeloma contributed to VRE acquisition.

Keywords Vancomycin resistance, Hematologic malignancies, Colonization, Risk factors

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BRIEF REPORT

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Risk factors for gut colonization with vancomycin-resistant enterococci among Bulgarian critically ill patients

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Abstract

Vancomycin-resistant enterococci (VRE) are recognized as important hospital pathogens which have become common in patients admitted to the intensive care units (ICUs). The purpose of this study was to evaluate the incidence of and the risk factors for colonization with VRE among ICU patients. A total of 91 patients who had duration of hospitalization more than 48 h and without infection caused by VRE or/and other microorganisms in the ICU at University Hospital, Pleven were screened for colonization with VRE. The following data were collected: demographic characteristics, clinical information and antimicrobials use. The statistical analysis was performed using SPSS version 27.0. Colonization with VRE was established in 22 patients and one was carrying two enterococcal species. A total of 23 VRE were isolated. The univariate analysis showed that the postoperative critical cares ($p < 0.001$), cardiovascular diseases ($p = 0.009$) and the presence of an endotracheal tube ($p = 0.003$) were risk factors for colonization with VRE. Also, the postoperative critical cares ($p = 0.021$) and cardiovascular diseases ($p = 0.018$) were confirmed as independent risk factor for VRE acquisition by multivariate analysis. The prevalence of VRE colonization among the ICU patients was relatively high (24.2%). Risk factors for acquisition of intestinal VRE were the postoperative cares, cardiovascular diseases and the presence of an endotracheal tube.

Keywords Vancomycin-Resistant Enterococci, Intensive care units, Risk factors

Main text

Vancomycin-resistant enterococci (VRE) are one of the multidrug-resistant pathogens with the highest hospital prevalence. Their ecological plasticity, ability to colonize the human intestinal tract, as well as prolonged decolonization thereafter are prerequisites for their widespread nosocomial dissemination [1]. The colonization with VRE precedes and plays a key role in the epidemiology of enterococcal healthcare-associated infections [2, 3]. The incidence of VRE colonization and infection is highest among patients with bone marrow transplantation and those who are treated in intensive care units (ICUs), while it is insignificant in immunocompetent individuals [4].

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Bilateral unusual branching pattern of the external carotid artery in a human cadaver

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Abstract: Comprehensive understanding of the variations in the branching of the external carotid artery (ECA) is essential to minimizing vascular complications during cranio-facial and neck surgical procedures. We demonstrate a rare case of unusual branching of ECAs in both carotid triangles and anomalous origin of the left ascending pharyngeal artery (APA) during dissection of embalmed cadaver. The right and left common carotid arteries (CCA) bifurcated at the level of the upper border of the thyroid cartilage. The right superior thyroid artery (STA) originated anterior to the carotid bifurcation (CB), while the left STA originated from the anterior aspect of the left CCA. The right ECA trifurcated into linguofacial trunk, APA, and distal ECA, 15.7 mm from CB. On the left side, lingual artery and APA arose as a short common linguopharyngeal trunk, 1.9 mm from CB. The left facial and occipital arteries originated anteromedially and posteriorly at the same level.

Key words: External carotid artery, Ascending pharyngeal artery, Linguofacial trunk, Superior thyroid artery, Linguopharyngeal trunk

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Introduction

The external carotid artery (ECA) with its branches is the major source for blood supply in the neck and head regions. Normally, it arises from the common carotid artery (CCA) together with the internal carotid artery (ICA) at the level of the superior border of the thyroid cartilage. Usually, the ECA gives eight branches: superior thyroid artery (STA), lingual artery (LA), and facial artery (FA) from its ventral surface; from its posterior surface arise the occipital artery (OA) and posterior auricular artery; the ascending pharyngeal artery (APA) from its medial surface; the maxillary artery and superficial temporal artery as terminal branches [1]. Despite

this classic textbook representation, numerous variations in the branching pattern of the ECA have been described [2–9]. The origin of ventral branches of the ECA in the form of common trunks, such as thyrolingual (TL), linguofacial (LFT), thyrolinguofacial (TLF), occipitoascendingpharyngeal, and occipitoauricular, has also been discussed [10, 11]. We demonstrate a rare case of unusual branching of the ECAs in both carotid triangles and anomalous origin of the left APA.

A comprehensive understanding of the variations in the branching of the ECA is crucial for minimizing vascular complications during surgeries (thyroidectomy, laryngectomy, tonsillectomy, glossectomy, etc.), ligating the ECA or its branches in severe epistaxis, arterial angiograms, embolization, and intra-arterial chemotherapy.

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Case Report

During routine academic dissection of the neck region of an embalmed female cadaver, the carotid triangles were

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Rare variation of the branches of the coeliac trunk and the common hepatic artery in human cadaver: a case report

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Background: The liver with its dual blood supply from the portal vein and the common hepatic artery (CHA) is unique among parenchymal organs. CHA accounts for around 30% of blood flow to the liver but has a central role in hepatobiliary perfusion.

Case presentation: We demonstrated a rare case of unusual branching of the CHA during routine dissection of an embalmed female cadaver. Measurements of the diameter and distance of origin of the branches were performed. The coeliac trunk presented with five branches – left gastric, splenic, CHA, right inferior phrenic, and accessory left hepatic arteries. The CHA penta-furcated into the left hepatic (LHA), middle hepatic (MHA), right hepatic (RHA), cystic (CA), right gastric (RGA), and gastroduodenal arteries (GDA). The first branch presented was RGA to the lesser curvature of the stomach. Instead of proper hepatic artery, three branches were observed – LHA, MHA, and posteriorly and between them, RHA. Inferiorly the GDA, with its diameter of 4 mm, represented a direct continuation of CHA. Accessory RHA artery was also identified branching from superior mesenteric artery.

Discussion: Variations of the hepatic arterial blood supply are seen in more than half of the population. The described variations were not previously identified according to the available literature.

Conclusion: Such complex variation, as the one presented in the current case, represents significant deviation from the norm. In the past, they were considered anatomical curiosities but now, in the era of complex liver surgeries and arterial-directed therapies, their relevance is being highly recognized.

Keywords: accessory right hepatic artery, case report, coeliac trunk, common hepatic artery, middle hepatic artery

Introduction

The liver is unique among parenchymal organs with its dual blood supply from the portal vein (PV) and the common hepatic artery (CHA). The CHA provides only around 30% of blood flow to the liver but has a central role in hepatobiliary perfusion. Normally, the coeliac trunk (CT) originates from the aorta as an unpaired visceral branch. It then trifurcates into the left gastric artery (LGA), the splenic artery (SA), and the CHA, which shortly after divides into the gastroduodenal artery (GDA) and

HIGHLIGHTS

- We demonstrated a rare case of unusual branching of the common hepatic artery during routine dissection of an embalmed female cadaver.
- The coeliac trunk presented with five branches – left gastric, splenic, common hepatic, right inferior phrenic, and accessory left hepatic arteries.
- The common hepatic artery penta-furcated, 23.9 mm from its origin from the coeliac trunk, into the left hepatic, middle hepatic, right hepatic, cystic, right gastric, and gastroduodenal arteries.
- Accessory right hepatic artery was also identified branching from superior mesenteric artery.

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the proper hepatic artery (PHA). The PHA divides into two main branches: the right hepatic (RHA) and the left hepatic arteries (LHA) supplying the eight segments of the liver. The right gastric artery (RGA) also usually originates from the PHA. The classical branching pattern of CHA was found in 55%–75.7% of the cases^[1–4]. Michels^[1] introduced a classification system that described the main variations of the vascular anatomy of the liver. This classification system was modified by Hiatt *et al*^[3] and supplemented by Koops *et al*^[2]. Despite such complex classifications, there were rare variations that did not fit any of them^[5–8]. Here, we present penta-furcation of the CHA which was not previously described and should be an extremely rare hepatic artery variant. Trifurcation and quadrifurcation of CHA were

NEUROSYSTEMS

Lateral regions of the rodent striatum reveal elevated glutamate decarboxylase 1 mRNA expression in medium-sized projection neurons

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Keywords: GAD1, GAD2, *in situ* hybridization, mouse, rat

Abstract

The GABA-synthesizing enzymes glutamate decarboxylase (GAD)1 and GAD2 are universally contained in GABAergic neurons in the central nervous system of the mouse and rat. The two isoforms are almost identically expressed throughout the brain and spinal cord. By using *in situ* hybridization, we found that the mouse lateral striatum concentrates medium-sized projection neurons with high-level expression of GAD1, but not of GAD2, mRNA. This was confirmed with several types of riboprobe, including those directed to the 5′-noncoding, 3′-noncoding and coding regions. Immunohistochemical localization of GAD1 also revealed predominant localization of the enzyme in the same striatal region. The lateral region of the mouse striatum, harboring such neurons, is ovoid in shape and extends between interaural +4.8 and +2.8, and at lateral 2.8 and dorsoventral 2.0. This intriguing region corresponds to the area that receives afferent inputs from the primary motor and sensory cortex that are presumably related to mouth and forelimb representations. The lateral striatum is included in the basal ganglia-thalamocortical loop, and is most vulnerable to various noxious stimuli in the neurodegeneration processes involving the basal ganglia. We have confirmed elevated expression of GAD1 mRNA, but not of GAD2 mRNA, also in the rat lateral striatum. Image analysis favored the view that the regional increase is caused by elevated cellular expression, and that the greatest number of medium-sized spiny neurons were positive for GAD1 mRNA. The GAD1 mRNA distribution in the mouse lateral striatum partially resembled those of GPR155 and cannabinoid receptor type 1 mRNAs, suggesting functional cooperation in some neurons.

Introduction

GABA is the best known inhibitory neurotransmitter in the adult mammalian brain, and is a key element in locomotion, reproduction, and learning. In the central nervous system, GABA is produced by glutamate decarboxylase (GAD). GAD exists as two isoforms, GAD1 and GAD2, each encoded by a different gene (Erlander *et al.*, 1991; Kaufman *et al.*, 1991; Soghomonian & Martin, 1998). GAD1 and GAD2 are present in most of the GABA-containing neurons, but the level of expression is often different (Erlander *et al.*, 1991; Esclapez *et al.*, 1993, 1994; Feldblum *et al.*, 1993). Different levels of GAD1 and GAD2, together with differences in cofactor interactions, might be related to distinctive functions within neurons (Kaufman *et al.*, 1991; Martin *et al.*, 1991; Battaglioli *et al.*, 2003).

Changes in GABA signaling appear to play a key role in pathological conditions such as Parkinson's disease, Huntington's disease, epilepsy, depression, anxiety and panic disorders. Interactions between dopamine and GABA play a pivotal role in the modulation of

motor behavior by the basal ganglia. In Parkinson's disease, the lateral striatum appears to be more severely depleted in dopamine and is among the earliest regions to be affected by the disease process (Kish *et al.*, 1988; Brück *et al.*, 2006; Nandhagopal *et al.*, 2009; Reetz *et al.*, 2009). Selective degeneration of lateral striatal medium spiny neurons is reported in Huntington's disease mice (Hodgson *et al.*, 1999; Denovan-Wright & Robertson, 2000).

In the striatum, two classes of medium-sized projection neuron receive widespread cortical afferents and maintain transient inhibitory effects upon their targets: one projecting to the globus pallidus (GP) through the enkephalin/GABA-containing neurons, and another projecting directly to the output station, the entopeduncular nucleus/substantia nigra pars reticulata, through the substance P (SP)/GABA-containing neurons (Gerfen, 2004). The levels of GABA and GAD enzymes required for the modulation of cortical activity might be different inside each class of projection neuron.

We have previously shown the preferential expression of GPR155 in the projection neurons, concentrated in the lateral striatum (Trifonov *et al.*, 2010). This pattern of distribution closely resembled that for cannabinoid receptor type 1 (CB1) (Hohmann & Herkenham, 2000). Previous studies in rats (Esclapez *et al.*, 1993, 1994) have shown in detail the comparative distribution and distinctive features of

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RESEARCH ARTICLE

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Glutamic acid decarboxylase 1 alternative splicing isoforms: characterization, expression and quantification in the mouse brain

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Abstract

Background: GABA has important functions in brain plasticity related processes like memory, learning, locomotion and during the development of the nervous system. It is synthesized by the glutamic acid decarboxylase (GAD). There are two isoforms of GAD, GAD1 and GAD2, which are encoded by different genes. During embryonic development the transcription of GAD1 mRNA is regulated by alternative splicing and several alternative transcripts were distinguished in human, mouse and rat. Despite the fact that the structure of GAD1 gene has been extensively studied, knowledge of its exact structural organization, alternative promoter usage and splicing have remained incomplete.

Results: In the present study we report the identification and characterization of novel GAD1 splicing isoforms (GenBank: KM102984, KM102985) by analyzing genomic and mRNA sequence data using bioinformatics, cloning and sequencing. Ten mRNA isoforms are generated from GAD1 gene locus by the combined actions of utilizing different promoters and alternative splicing of the coding exons. Using RT-PCR we found that GAD1 isoforms share similar pattern of expression in different mouse tissues and are expressed early during development. Quantitative RT-PCR was used to investigate the expression of GAD1 isoforms and GAD2 in olfactory bulb, cortex, medial and lateral striatum, hippocampus and cerebellum of adult mouse. Olfactory bulb showed the highest expression of GAD1 transcripts. *Isoforms 1/2* are the most abundant forms. Their expression is significantly higher in the lateral compared to the medial striatum. *Isoforms 3/4, 5/6, 7/8* and *9/10* are barely detectable in all investigated regions except of the high expression in olfactory bulb. When comparing GAD1 expression with GAD2 we found that *Isoforms 1/2* are the predominant isoforms. *In situ* hybridization confirmed the predominant expression of *Isoforms 7/8* and *9/10* in the olfactory bulb and revealed their weak expression in hippocampus, cerebellum and some other areas known to express GAD1.

Conclusions: Generation of ten splicing isoforms of GAD1 was described including two so far uncharacterized transcripts. GAD1 splicing isoforms producing the shorter, enzymatically inactive GAD25 protein are expressed at very low level in adult mouse brain except in the olfactory bulb that is associated with neurogenesis and synaptic plasticity even during adulthood.

Keywords: GAD1, GAD2, Alternative splicing, Quantitative RT-PCR, *in situ* hybridization

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A STUDY ON THE ROLE OF THROMBOPHILIC GENETIC DISORDERS AS A RISK FACTOR FOR THROMBOTIC COMPLICATIONS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

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Summary

Myeloproliferative neoplasms (MPN) are haematological diseases, characterized by clonal hematopoiesis. Hemostasis abnormalities are among the most critical and frequent complications, affecting the quality of life and a possible reason for death. Thrombotic complications are common and multifactorial. Our aim was to study some genetic thrombophilia factors – Factor V Leiden (FVL), G20210A mutation in prothrombin gene (PR G20210A) and PLA2 allele polymorphism of glycoprotein IIIa gene (GPIIIa gene), and their frequency and association with thrombotic risk in both Philadelphia-positive and Philadelphia-negative MPN – chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary and secondary myelofibrosis (MF). In our patient population, PLA2 allele polymorphism of GPIIIa gene proved to be the most common and significantly associated with thrombotic complications – 26.85% of our patients were carriers, and 24.14% of them reported thrombotic complications.

Key words: genetic thrombophilia, myeloproliferative neoplasms, thrombotic complications

Introduction

Myeloproliferative neoplasms (MPN) are clonal disorders of hematopoiesis, originating from stem cells. They are characterized by the accumulation of cells of different cell lines in the bone marrow, peripheral blood and spleen, as well as in other organs, that are prone to terminal differentiation [1]. The term covers chronic myelogenous leukaemia (CML), myelofibrosis (MF), polycythemia vera (PV), essential thrombocythemia (ET), as well as some rare conditions like chronic eosinophilic leukaemia, mastocytosis and others [2].

These conditions are divided into two main groups: Philadelphia-positive and negative, depending on Philadelphia chromosome carrier - t(9;22)(q34;q11). CML is Philadelphia-positive, while PV, ET and myelofibrosis (MF, primary and secondary) are Philadelphia-negative. The aetiology of these diseases is still not clearly defined, but many studies have determined the main pathogenic changes. Accumulation of terminally differentiated cells displaces normal hematopoiesis (clonality), resulting

Research Article

Design of primers and optimization of PCR conditions for the detection of alternatively spliced isoforms of mouse ChAT mRNA

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Summary

Acetylcholine plays an important role as a neurotransmitter in the central and peripheral nervous systems. Its biosynthesis is catalyzed by choline acetyltransferase (ChAT). In mice, the ChAT gene has three 5'-noncoding (R, N, and M) and 14 coding exons, from which seven mRNA isoforms (M, N1, N2, R1, R2, R3, and R4) are transcribed. They differ in the 5'-noncoding ends and encode the same protein (common ChAT). An additional isoform that lacks coding exons from 5 to 8 encodes a smaller protein (peripheral ChAT). This research aimed to design and check the specificity of primers targeting R3, N1, N2, M, peripheral ChAT, and common ChAT isoforms. The optimal PCR conditions and specificity of the primers were tested in a series of PCR reactions. Specially designed DNA fragments or plasmids containing isoform-specific nucleotide sequences were used as templates. The appropriate annealing temperature, which yields sufficient specificity for each tested primer pair, was as follows: R3 – 60 °C; N1 – 63 °C; N2 – 65 °C; M – 65 °C; peripheral ChAT – 65 °C and common ChAT – 63 °C.

Key words: Choline acetyltransferase, primer, polymerase chain reaction, alternatively spliced isoforms



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Introduction

Most protein isoforms arise from alternative splicing of the primary RNA transcripts. Splicing provides an extensive collection of protein isoforms that display different properties and serve various functions (Graveley 2001; Liu et al. 2022). This way, alternative splicing is one of the main mechanisms to expand biological diversity. As a neurotransmitter, acetylcholine (ACh) has important functions both in the central (Armstrong et al. 1983; Li et al. 2018) and in the peripheral nervous system (Tooyama and Kimura 2000; Bellier and Kimura 2007). Choline acetyltransferase (acetyl-CoA, choline O-acetyltransferase, ChAT), a cytoplasmic enzyme in cholinergic neurons, catalyzes the biosynthesis of ACh from acetyl-coenzyme A and choline. Then, the vesicular acetylcholine transporter (VAChT) translocates ACh into the synaptic vesicles (Brown 2006, 2019). In the brain, ACh is involved in fundamental processes like arousal, sleep, learning, memorizing, cognition, and movement (Obermayer et al. 2017; Solari and Hangya 2018), whereas in the spinal cholinergic motor neurons it is the neu-



ASSOCIATION OF THE LEPTIN RECEPTOR Q223R GENE POLYMORPHISM AND FASTING PLASMA GLUCOSE LEVELS IN NORTH BULGARIAN SUBJECTS

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ABSTRACT

Purpose: Obesity, diabetes, and insulin resistance are growing public health concerns, and their incidences are constantly increasing. Imbalances in whole body glucose and insulin homeostasis are also closely linked to the trend of increased obesity. A number of single nucleotide polymorphisms (SNPs) of the coding region of the leptin receptor (LEPR) gene have been described and associated with obesity measures in different ethnic groups. In Q223R polymorphism, there is a non-conservative A to G substitution at codon 223, resulting in the replacement of glutamine with arginine amino acid. This changes the binding affinity of LEPR and impairs leptin signaling. The aim of the present study was to investigate the association between the Q223R SNP and obesity related anthropometric and biochemical parameters in the North Bulgarian population.

Material/Methods. The study included 156 subjects. Body weight, body mass index and blood pressure were measured, and levels of fasting plasma glucose (FPG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) were determined. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism.

Results. The results showed a significant association between the GG genotype of the LEPR Q223R polymorphism and elevated FPG levels. However, no association was found between LEPR genotypes and obesity status, HDL, LDL, or TC levels in North Bulgarian participants.

Conclusions. The homozygous GG genotype of the LEPR Q223R polymorphism is associated with higher fasting plasma glucose levels and may be a predisposing factor for type 2 diabetes mellitus.

Keywords: Leptin receptor, *LEPR* Q223R polymorphism, obesity, fasting plasma glucose,

INTRODUCTION

Metabolic complications such as obesity, diabetes, and insulin resistance are growing public health concerns, and their incidences are continuously increasing [1]. Imbalances in whole body glucose and insulin homeostasis are also closely linked to the trend of increased obesity [2]. Therefore, understanding the molecular and cellular mechanisms underlying energy balance, glucose, and insulin homeostasis is crucial for the development of new strategies for the prevention and treatment of obesity and diabetes [3].

Leptin is an adipocyte-secreted hormone that is among the indicators of the energy and nutritional status of the brain and was found to be involved in the development of obesity and its related traits [2, 4]. Leptin exerts its effect via the leptin receptor (LEPR) encoded by the diabetes gene (*db*) located in humans in chr1p31 [5]. It was found to be expressed by neurons in numerous hypothalamic nuclei such as area hypothalamica lateralis, area hypothalamica intermedia, nucleus ventromedialis and nucleus arcuatus s. infundibularis [3]. These nuclei are regulatory centers of energy homeostasis and appetite.

Mutations of the *LEPR* gene were associated with monogenic forms of severe early onset obesity, hyperphagia [6]; impaired glucose tolerance and type 2 diabetes [7]; and insulin resistance [8]. Functionally and clinically, coding polymorphisms in *LEPR* can alter the receptor's structure, affecting leptin binding, receptor activation, and downstream signaling pathways that regulate appetite and metabolism. They have been linked to an increased risk of obesity, insulin resistance, type 2 diabetes, and metabolic syndrome, although the strength of these associations can vary depending on ethnicity and environmental factors. Importantly, variants that reduce receptor function often cause elevated circulating leptin levels but a diminished physiological response to leptin, which is a hallmark of leptin resistance commonly observed in obesity. Understanding these polymorphisms is valuable for developing personalized approaches to obesity treatment and metabolic disease management, as well



Article

Association of Adiponectin Gene Polymorphism rs266729 in Obese People with Type Two Diabetes Mellitus in North Bulgaria's Population

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Abstract

The alarming surge in obesity rates represents a critical health concern in developed nations. This condition is associated with several health issues grouped under the term metabolic syndrome. This alarming data mandates an investigation into the causes and risk of this syndrome. One category of causes is genetic factors. The adiponectin gene has been subject to several studies exploring its single nucleotide polymorphisms, especially rs266729, across diverse populations. This study included 156 subjects. Body weight, body mass index, and blood pressure were measured, and levels of fasting plasma glucose (FPG), total cholesterol, triglycerides, HDL, and LDL were determined. Genotyping was performed. There was significant association of rs266729 polymorphism with obesity and type 2 diabetes mellitus (T2DM), with notably higher frequency of the G allele among these subjects. Additionally, the results demonstrated significant influence on the detected levels of HDL. Individuals carrying the homozygous (GG) and heterozygous (CG) genotypes exhibited a three-fold and two-fold increased risk of elevated FPG levels, respectively. There was also a significant association of these genotypes with T2DM when compared with the wild-type genotype (CC). These observations strongly indicated a potential involvement of the adiponectin gene polymorphism rs266729 in the pathogenesis of T2DM among subjects from North Bulgaria.

Keywords: adiponectin; polymorphism; obesity; type 2 diabetes mellitus

1. Introduction

The alarming surge in obesity rates represents a critical health concern in developed nations. This condition is intricately associated with an array of health issues grouped under the term “metabolic syndrome”, encompassing the development of insulin resistance, type 2 diabetes mellitus (T2DM), cardiovascular complications, and fatty liver disease [1]. The intricate interplay between obesity and T2DM stems from their shared link with insulin resistance, a key factor in their pathogenesis [1]. At the forefront of health challenges in the 21st century, T2DM constitutes a substantial majority (90–95%) of diabetes cases. It spans a spectrum from predominantly insulin resistance with relative insulin deficiency to scenarios characterized by an insulin secretory defect alongside insulin resistance [2]. Presently afflicting a staggering 317 million individuals worldwide, in Bulgaria alone it impacts around 9.9% of the adult population, totaling approximately 519,300 individuals [3]. This escalating prevalence correlates with the rapid upsurge in obesity rates witnessed across various countries [4]. The concerning data mandates a comprehensive investigation of the causes and risks associated with metabolic syndrome and specifically T2DM. One



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Women's Invaluable Contribution in the Development of Anatomical Knowledge and Education through the Centuries

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Stereotypes and prejudice have long been significant barriers to women's participation in the development of anatomical knowledge and education. Despite these challenges, generations of women have persevered, overcoming numerous obstacles to establish themselves in the field. The history of anatomy is often viewed as male-dominated, yet women have made significant contributions, even when societal norms or legal restrictions sought to limit their involvement. Women excelled as prosectors, anatomists, researchers, and educators – even when such practices were viewed as scandalous or were prohibited. This historical review aims to shed light on the contributions of female anatomists, who were not just scientists but also philosophers, innovators, and visionaries. By documenting their achievements, we seek to raise awareness of their invaluable role in shaping the field of anatomy. Recognizing the history of these women is crucial in understanding both the progress made and the ongoing need for gender equality in science and medicine.

Key words: history of medicine, women anatomists, history of anatomy, dissection, wax modeling

Anatomy is a foundational subject for many fields of study. A deeper exploration of this discipline often inspires admiration in those who choose to pursue it further. However, it is not only the complexity and significance of the subject itself that draws admiration, but also the discoverers who showed courage and boldness in their exploration. As a mark of respect, many anatomical structures and anomalies have been named after their discoverers [12, 21]. What stands out, however, is that most of the well known anatomical terms named after scientists are associated with male names. This raises important questions: What has been the role of women in anatomy and anatomy



Review

Molecular Diversity and Functional Implications of Mammalian Choline Acetyltransferases in Neuronal and Non-Neuronal Cells

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Abstract

Acetylcholine (ACh) is the first identified neurotransmitter and an evolutionarily conserved signaling molecule. Although its role in classical synaptic transmission within the central and peripheral nervous systems has been extensively studied, growing evidence indicates that cholinergic signaling extends beyond neuronal synapses and operates in a broad range of non-neuronal cells. Thus, the cholinergic system represents a complex and widely distributed signaling network with both neuronal and non-neuronal components. Within the nervous system, cholinergic neurons display marked molecular heterogeneity, largely driven by the genomic organization and alternative splicing of the choline acetyltransferase (ChAT) gene. Distinct ChAT mRNA splice variants contribute to region- and cell-type specific cholinergic phenotypes in central and peripheral neurons, including the enteric nervous system, which exemplifies a highly autonomous peripheral cholinergic network. Beyond the nervous system, non-neuronal cholinergic signaling has been identified in epithelial, cardiac, immune, and other cell types, where ACh acts as an autocrine and paracrine regulator of key physiological processes. This review summarizes current knowledge on ACh biosynthesis, focusing on ChAT and its splice variants as molecular determinants of cholinergic diversity and function across neuronal and non-neuronal contexts.

Keywords: acetylcholine; cholinergic neurons; choline acetyltransferase (ChAT); enteric nervous system (ENS); mRNA splice variants

1. Introduction

Acetylcholine (ACh) is the founding member of the class of biochemicals known as neurotransmitters and is widely distributed in both neuronal and non-neuronal cells. Early conceptual foundations for chemical signaling were laid by John Newport Langley [1], who proposed the existence of “receptive substances” (later termed receptors) on target cells. The physiological role of ACh as a neurotransmitter was initially suggested by Henry Dale, but direct evidence came from a series of experiments. Otto Loewi’s 1921 vagus nerve experiment demonstrated release of a diffusible substance (“Vagusstoff”), though the original work was methodologically complex and required subsequent clarification by Loewi and Navratil, who later identified the substance as ACh and characterized its enzymatic degradation by cholinesterase [2]. Conclusive proof that ACh functions as a transmitter was provided by Dale, Feldberg, and Vogt, who showed its presence and release at nerve endings [3]. In recognition of these foundational contributions, Dale and Loewi were jointly awarded the Nobel Prize in 1936 [4,5].



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**МОРФОЛОГИЧНА ХАРАКТЕРИСТИКА НА
МАКРОНЕВРОНИ В ЗОНАТА НА ВЕНТРАЛНОТО
СЛУХОВО ЯДРО ПО ВРЕМЕ
НА ПОСТНАТАЛНИЯ ПЕРИОД**

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**MORPHOLOGICAL CHARACTERISTICS OF
MACRONEURONS IN THE REGION OF THE VENTRAL
COCHLEAR NUCLEUS DURING
THE POSTNATAL DEVELOPMENT**

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ABSTRACT We have studied two types of macroneuronal cell bodies: globular and multipolar in the region of the Ventral Cochlear Nucleus (VCN), during the different stages of the postnatal development. Twelve white rats (Wistar) ranging in age from 15 – 70 day after birth were used in this study for electron microscopic investigation. During 15 – 16 postnatal day we recognized “immature” form of this cells. The neurons have been differentiated according to their specific morphology of the pericarya, nuclei, rough endoplasmic reticulum and synapses from cochlear origin. Elucidation of the ultrastructure of synaptic organization in the region of VCN is a morphological basis for correlation of these data with immunocytochemical and neurophysiological characteristics of described neurons.

Key words: ventral cochlear nucleus, synapses, globular cells, multipolar cells

ВЪВЕДЕНИЕ

Влакната на слуховия нерв инервират вентралното слуховото ядро по високо организиран и честотно специфичен начин известен като тонотопия. Тонотопичната инервация намира израз в определянето на т.нар. изофреквентни полета, в които два невронални типа са широко представени – глобуларни и мултиполярни разграничаващи се според характеристиката на перикариона и синаптичната организация.

КЛИНИЧЕН ПОДХОД В СЪВРЕМЕННОТО ПРЕПОДАВАНЕ НА АНАТОМИЯ – ИДЕИ И ПЕРСПЕКТИВИ

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Keywords: cutaneous melanoma, risk factor, non-solar ultraviolet radiation

Медицинският университет в гр. Плевен е основан през 1974 г. като факултет на Медицинска академия – София. Той е един от най-младите, но вече достатъчно утвърдени медицински висши училища. Програмата по анатомия през тези 30 години не е коренно променяна. От 2000 г. в МУ – Плевен се експериментират нови методи в преподаването на предклиничните дисциплини.

Преподаваната в България класическа дескриптивна анатомия е показала в годините своята ефективност и стойност. Усвоена качествено в курса на обучение, тя дава солидна база, върху която да се надстроят клиничните знания. Големото предимство на този подход е акцентирането върху детайлното изучаване на всяка отделна структура на макро- и микроскопско ниво. Тази безспорно ценна практика, осигуряваща задълбочено и подробно познаване на биологичните обекти в днешно време, започва да става недостатъчна.

Възниква необходимостта подробно изученият с методите на класическата анатомия “мъртъв” орган да бъде представен със своя нормален анатомичен образ в живия индивид чрез възможностите на съвременните образни технологии [1,4]. Така компютърната томография /КТ/ и магнитно-резонансната томография /МРТ/ позволяват своеобразна “виртуална” дисекция. Давайки изображение съвсем близко до реалния дисекционен срез в триизмерни равнини, тези методики

Clinical approach in teaching anatomy – ideas and perspectives

Computer tomography, magnetic resonance image and other interventional methods are widely used in modern medicine and impose actualization and clinical approach in teaching anatomy. The results of inquiry after workshops of topographic anatomy with elements of operative surgery and image diagnostic, prompt the new aspects in teaching anatomy: integration of the anatomy with clinic, early acquiring of practical surgical skills in dissections and the paramount place of anatomy in continuing medical education. Our future plans are to emphasize on teaching image anatomy and to enhance students' interest in dissections by introduction of "clinical tasks".

стават необходимото свързващо звено между класическата анатомия и клиничната. Това е анатомията, с която започват да работят студентите, прекращвайки в клиниката. Тогава възниква необходимостта да се използват натрупаните теоретични анатомични знания в реални условия. Познаването на нормалния морфологичен образ на органите на макроскопско ниво, визуализиран чрез модерните технологии за образна диагностика, е необходимото и задължително условие за усвояване и различаване на патоморфологичните изменения при различните заболявания.

МОГАТ ЛИ АКТИВНИТЕ ФОРМИ НА ОБУЧЕНИЕ ДА НАПРАВЯТ АНАТОМИЯТА ПО-ИНТЕРЕСНА

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CAN ACTIVE LEARNING ELEMENTS MAKE ANATOMY MORE INTERESTING

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ABSTRACT

Anatomy is the main basic discipline in medicine. The huge amount of facts and Latine terms make it difficult and boring to study for 2/3 of students.

The aim of our study was to assess the results of implementing an active learning elements in teaching.

In the course of internal organs students were divided in 2 groups: group of active learning and control– with classic learning. The first group were given short clinical cases on which students should study and try to explain the symptoms with the acquired anatomy knowledge. The results were assessed by interim and final tests and questionnaires.

Key words: active learning methods, anatomy

Анатомията е основна предклинична дисциплина в обучението по медицина. В Медицинския университет – Плевен тя се изучава 3 семестъра (в I и II курс) с общ хорариум 334 учебни часа (19 кредита). Преди навлизането си в клиниката в III курс, студентите не могат да оценят важното място на анатомичните знания в лекарската практика [4, 8]. Класическата програма не обвързва преподаването по анатомия с клиниката и понеже тя е обемиста наука с много фактология и латински термини, става трудна и скучна за учене [5, 10, 11]. Затова в много медицински училища по света се въвеждат нови, насочени към студента методи за обучение, стимулиращи активното учене [3-11]

През 2003 г.проведохме анкета със студенти от V и VI курс и специализанти, в която се интересувахме от мнението им за методите на преподаване по анатомия, мястото на анатомичните познания в клиниката и процеса на учене по дисциплината. Анкетата показва, че студентите осъзнават важността на знанията по анатомия, но 2/3 предпочитат

LIGHT MICROSCOPIC IDENTIFICATION AND NEURONAL MORPHOLOGY OF THE DORSAL NUCLEUS OF THE LATERAL LEMNISCUS IN THE RAT

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Summary:

Introduction: The nuclei of the lateral lemniscus (ventral, intermediate and dorsal) play an important role in the auditory system. They are the main source of inhibitory influence in the auditory brainstem of mammals. The investigation of cytoarchitecture and connectional properties of these nuclei is complicated because of their position within the fiber tract of the lateral lemniscus (LL).

Aim: The goal of the present study is to identify the boundaries and neuronal cell types in the dorsal nucleus of the lateral lemniscus (DLL).

Material and Methods: Three Wistar rats, under deep ether anaesthesia, were perfused through the heart with 4 % paraformaldehyde in 0,1 M phosphate buffer (pH 7,4). After progressive dehydration and embedding in paraffin, 5 μ m thick sections were cut and stained with cresyl violet.

Results and Discussion: As seen in Nissl stained material, the dorsal boundary of the DLL is ventrolateral border of the inferior colliculus; the medial border is formed by the cuneiform nucleus; the lateral - by the nucleus sagulum. The rostral boundary of the DLL is the microcellular tegmental nucleus and the caudal border of the DLL is the lateral parabrachial nucleus.

In the region of the DLL can be distinguished four different neuronal types, according to the following morphological criteria: size, shape and contour of the cell body, the size and position of nucleus and nucleolus, the Nissl pattern of the cytoplasm. Type I neurons are large and multipolar. Type II neurons are large bipolar cells orientated lateromedially. Type III neurons are medium in size and their soma is round or ovoid. Type IV neurons are small and round with scant cytoplasm and are also the least common neuronal type of the DLL.

The parallel morphometric and statistical analysis verifies the light microscopic classification of neuronal cell types of the DLL.

Key words: the dorsal nucleus of the lateral lemniscus, light microscopy, rat

Introduction:

The nuclei of the lateral lemniscus (LL) are an important part of the auditory system because they are the main source of inhibitory influence in the auditory brainstem of all mammals (Malmierca, '2003). According to some morphological and physiological criteria there could be distinguished three separate nuclei in the region of the lateral lemniscus (LL) – ventral nucleus of the lateral lemniscus (VLL), intermediate nucleus of the lateral lemniscus (ILL) and dorsal nucleus of the lateral lemniscus (DLL). The dorsal nucleus of the lateral lemniscus (DLL) is a group of neurons scattered within the fibers of the LL and located immediately ventral to the inferior colliculus (Zook and Casseday, '82; Adams and Mugnaini, '84; Bajo et al., '93; Merchan et al., '97; Paxinos

and Watson, '86; Malmierca, '2003). The boundaries of the DLL are not well defined, although in Nissl - stained sections through DLL, large intensely stained neurons are easily distinguishable (Adams and Mugnaini, '84, Bajo et al., '93).

Several neuronal types have been described, depending on the species and the criteria used for cell classification. Kane and Barone (1980) described nine types, according to the cell size and shape, in Nissl and Golgi stained material in the cat. Based on electron microscopic and Nissl stained sections have been described three neuronal types (Shneiderman et al., '88; Oliver and Shneiderman, '89). In mouse, Iwahori (1986) described three types of neurons according to the size and shape of their soma and the morphology



Cholinergic Fibers and Muscarinic Receptors in the Central Auditory System

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中枢聴覚系のコリン線維とムスカリン性受容体

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Abstract:

It was reported that central auditory dysfunction was evident in subjects with even mild cases of Alzheimer's disease. Major auditory stations in diseased brain manifested marked degenerative changes. This communication briefly summarized our morphological data on the distribution of cholinergic fibers and receptors in the ascending auditory stations of mouse brain. We revealed considerable involvements of m2 and m3 muscarinic receptors in several auditory stations, providing some morphological support for the cholinergic projections and receptors available to the auditory system.

Keywords: Cholinergic fibers, Muscarinic receptors, Auditory stations, Vesicular acetylcholine transporter,
Nucleus of the brachium of the inferior colliculus, Auditory-visual communication

要旨:

アルツハイマー病では中枢聴覚系の機能障害を示唆する症状を合併する 경우가少なくない。また細胞変性等の病変が疾病脳の聴覚上行路各部位にも確認されている。本研究ではマウス脳のこれらの部位におけるコリン線維とムスカリン性受容体 (m2, m3 タイプ) の発現分布密度を測定した。聴覚上行路諸核で、これらコリン線維・受容体を介する情報伝達・変換が聴覚の伝達に一定の意義を有することが、本研究から強く示唆された。

キーワード: コリン線維, ムスカリン性受容体, 聴覚経路, 小胞アセチルコリントランスポーター, 下丘腕核,
聴覚・視覚機能連関

The processing of sound information by the neurons of the central auditory system, initiating from the cochlear hair cells, is under constant modulation of their inhibitory and excitatory projection systems. There are several lines of evi-

dence that cholinergic fibers, forming cholinergic synapses with these neurons, might modulate sound processing in the central auditory stations via typical cholinergic receptors, coupling it with behavioral arousal, attention and cognitive

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Аномалията на Кимърли – едно подценявано клинично състояние



Атлас в норма - изглед отгоре

(Снимков материал и прешлени: авторски)



Атлас в норма - страничен изглед (отдясно)

Аномалията на Кимърли, известна още като пръстеновиден или дъговиден отвор (лат. Foramen arcuatum), представлява една от многото аномалии, засягащи първия шиен прешлен – атлас.

Атласът е неразделен компонент от свързването на черепа с гръбначния стълб. Той е пръстеновиден прешлен, разположен под тилната кост и е в тясна връзка с прешленната (вертебрална) артерия, подтилния (субокципитален) нерв и други важни анатомич-

ни структури. Състои се от предна и задна дъга, свързани помежду си в т. нар. странична костна маса. По горната повърхност на последната, атласът образува става с тилната кост, в която се извършват навеждане на главата напред, назад и встрани. Вертебралната артерия (заедно с подтилния нерв) обичайно заляга в бразда, разположена върху задната дъга на атласа. Тя води началото си от подключичната артерия, изкачва се във възходяща посока, защитена в добре оформен костен канал, заляга в браздата на атласа

Анатомично проучване върху задното мостче (ponticulus posticus) от костни препарати в катедра „Анатомия, хистология, цитология и биология“ при Медицински университет – Плевен

Задното мостче или понтикулус постикус (лат. ponticulus posticus – задно мостче) е анатомична вариация на първия шиен прешлен – атласа (лат. atlas). То се описва като пълен или непълен костен мост, започващ от страничната костна маса и насочващ се към задната дъга на атласа. Понтикулус постикус покрива браздата за прешленната артерия, превръщайки я в костен пръстен или канал, през който преминават прешленната артерия и вена, периаартериалното симпатиково сплетение, както и подтилния нерв. Отворът, който се загражда от задното мостче и задната дъга е известен още като дъговиден отвор на атласа (лат. - foramen arcuatum или foramen arcuale), горен ретроартикуларен отвор или канал, както и вари-

ация или аномалия на Кимърли.

В зависимост от степента на вкостяване на задното мостче, първият шиен прешлен може да се раздели в шест класа:

При клас I се забелязва отпечатък от прешленната артерия върху задната дъга.

При клас II отпечатъкът е по-дълбок, отколкото този при клас I.

При атласи, класифицирани като клас I и клас II, липсва развитие на



Бразда за прешленната артерия

Първи шиен прешлен (атлас), Клас I. Изглед от страни.



Бразда за прешленната артерия

Първи шиен прешлен (атлас), Клас I. Изглед отзад.

GLOMUS COCCYGEUM - АНАТОМИЧЕН ПРЕГЛЕД, ДИАГНОСТИЧНО И ТЕРАПЕВТИЧНО ЗНАЧЕНИЕ

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GLOMUS COCCYGEUM - ANATOMICAL OVERVIEW. DIAGNOSTIC AND THERAPEUTIC SIGNIFICANCE

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ABSTRACT: The glomus body is a microanatomical structure most commonly found in the reticular dermis of the distal extremities, particularly in the nail bed, fingertip, and palm. It is a specialized arteriovenous anastomosis that is involved in thermoregulation and blood circulation. One unusual site where glomus bodies have been identified is the lumbosacral and pericoccygeal region, the so-called glo-mus coccygeus.

It corresponds to a complex anastomosis between the middle sacral artery and vein. Each glomus body consists of several afferent arterial segments branching into preglomus arterioles, which continue as anastomoses, opening into the venous part of the blood stream. These anastomoses with an irregular lumen are known as Sucquet-Hoyer ducts. They are surrounded by epithelioid glomus cells, all of which lie within a fibrous stroma containing sympathetic nerve fibers. Several subtypes of glomus coccygeum have been described on light microscopic preparations: "glomus-cell nodular dominant type", "intermediate mixed type" (nodular and vascular) and "vascular-dominant type with scattered individual glomus cells".

This specialized arteriovenous anastomosis is considered a non-pathological rudimentary structure because the genetic structures that make it up rarely show a tendency towards malignant changes such as hyperplastic and neoplastic proliferation. Although no malignant differentiation of the structure is observed, the glomus coccygeum often mimics a glomus tumor, the object of attention of pathologists and surgeons. Glomus coccygeum is also associated with coccygodynia, a painful syndrome affecting the coccygeal region and causing diagnostic problems for pathologists unfamiliar with this entity.

Key words: glomus coccygeus, Sucquet-Hoyer canals, glomus tumor, coccygodynia

Въведение

Glomus coccygeum (GC), от лат. „кълбо прежда“ се отнася към гломусните телца, представляващи артерио-венозни анастомози, принадлежащи териториално и функционално към терминалното съдово русло. Типичните анастомози или шънтове привеждат артериалната кръв директно от артериалното към венозното русло (Овчаров В., Ванков В., 2019). Артерио-венозните анастомози в

Research Article

Adipose Tissue, Leptin Signaling, and Adiponectin in Obesity and Type 2 Diabetes Mellitus

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Abstract

The global rise in obesity represents a major public health challenge and is closely associated with the increasing prevalence of metabolic syndrome and type 2 diabetes mellitus (T2DM). Obesity is characterized by excessive accumulation of adipose tissue, which contributes to the development of insulin resistance, chronic low-grade inflammation, and multiple metabolic complications. T2DM accounts for the majority of diabetes cases and is a complex metabolic disorder involving both impaired insulin action and β -cell dysfunction. Its growing incidence parallels the increasing rates of obesity worldwide.

Adipose tissue is now recognized as a metabolically active endocrine organ rather than a passive energy reservoir. It plays a central role in energy homeostasis through lipid storage and the secretion of adipokines, including leptin and adiponectin, which are critical regulators of metabolic processes. Leptin is a key hormone involved in appetite regulation and energy expenditure, acting primarily through hypothalamic signaling pathways such as JAK/STAT, PI3K/Akt, MAPK, and AMPK. However, in obesity, elevated leptin levels fail to exert their expected physiological effects due to the development of leptin resistance, which is associated with disruptions in intracellular signaling, inflammation, and cellular stress responses.

In contrast, adiponectin exerts insulin-sensitizing, anti-inflammatory, and anti-atherogenic effects, primarily through activation of metabolic signaling pathways that enhance glucose utilization and fatty acid oxidation. Its circulating levels are inversely correlated with adiposity and are reduced in obesity and T2DM, contributing to metabolic dysregulation.

Understanding the molecular interplay between adipose tissue, leptin signaling, and adiponectin is essential for elucidating the pathogenesis of obesity-related metabolic disorders and for developing targeted therapeutic strategies.

Introduction

The alarming rise in obesity prevalence has generated significant concern among developed nations. This condition is associated with a wide spectrum of metabolic disorders collectively referred to as metabolic syndrome, including insulin resistance, type 2 diabetes mellitus, cardiovascular complications, and liver disease [1]. The close relationship between obesity and T2DM arises primarily from insulin resistance, which represents a central pathogenic mechanism in both conditions [1].

Type 2 diabetes mellitus accounts for approximately 90–

95% of all diabetes cases and encompasses a heterogeneous spectrum of metabolic disturbances, ranging from predominant insulin resistance with relative insulin deficiency to conditions characterized by impaired insulin secretion in combination with insulin resistance [2]. At present, approximately 317 million individuals worldwide are affected, with 9.9% of the adult population in Bulgaria, corresponding to approximately 519,300 individuals, diagnosed with T2DM [2]. This increase correlates strongly with the rapid rise in obesity rates observed in most countries [2].

The etiology of T2DM is multifactorial and includes genetic, environmental, and metabolic components. Advances in

More Information

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Keywords: Adipose tissue; Leptin signaling; Adiponectin; Insulin resistance; Type 2 diabetes mellitus





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Служебна бележка

Настоящата служебна бележка се дава в удостоверение на това, че доклад със заглавие:

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Сборникът с доклади е депозиран за рефериране в Националния референтен списък на съвременни български научни издания с научно рецензиране (<https://nrs.nacid.bg/register/search>).

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Председател на СУБ – Клон Плевен



Контракцията на стволовите клетки зависи от композицията на извънклетъчния матрикс

Цветан Попов, Стефан Трифонов

Stem cell contraction depends on matrix composition

Cvetan Popov, Stefan Trifonov

Abstract: *Collagen contraction is a general feature of the cells within the tissues. It represents their ability to remodel and adapt the matrix to their needs. This ability however, grants a significant challenge for the development of artificial tissues, especially when the aim is development of connective tissue that consists largely of extracellular matrix, dominated by the collagens. We investigated the contractile ability of adipose derived stem cells in two different collagen compositions – only collagen type I and mixture of collagen type I and type II. As main tool we used 3D collagen constructs (gels). We analyzed their remodeling ability over a period of 7 days as we also seeded the 3D gels with two different cell concentration - low and high. After the 7 days period, the superior contraction of collagen by the cells was observed in the mixed collagen type I and type II gel. Furthermore, number of cells has also an impact as the high number of cell demonstrated a faster remodeling capability of the extracellular matrix. Hence, we concluded that combination of the two collagens significantly boosted the stem cells ability to contract the matrix. We believe that our study might contribute into the understanding of the complex niche of cell-to-matrix interactions and could benefit future efforts in development of artificial tissues.*

Keywords: stem cells, collagen matrix, cell contraction;

INTRODUCTION

Stem cells are the major tool of regenerative medicine. Since their discovery [1] there is significant work done to understand their characteristics. Stem cells are cells that can adhere to different surfaces, can rapidly divide, express specific surface receptors (common CD73, 90 and 105) and are able to differentiate to minimum 3 different lineages (osteoblasts, chondrocytes and adipocytes). Furthermore, they are able to generate specific tissues in vivo.

With the development of the 3D tissue cultures due to ambition of the creation of artificial tissue, it became apparent the knowledge of the 2D cell cultures was mostly not applicable in complicated 3D conditions. Cells in a 3D environment were experiencing different stress factors like compression, weight of the tissue and reduced access to nutrition and oxygen. They also had different connections to the matrix and to the neighbouring cells, and were able to interact, remodel and replace the surrounding extracellular matrix [2]. Often, produced 3D cultures constructs were significantly different to the initial casted tissue [3] as they differ in size, cell density and matrix composition. This is caused by the specific cell ability named matrix remodeling. Simplified, matrix remodeling is a common cell ability to



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с автор: доц. д-р **Стефан Венелинов Трифонов, д.м.**

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Комуникация между ентералната нервна система и чревния микробиом

Десислава Маринова¹, Стефан Трифонов²

Communication between the Enteric Nervous System and the Gut Microbiome

Desislava Marinova¹, Stefan Trifonov²

Abstract:

The enteric nervous system (ENS) is a distinct component of the autonomic nervous system, comprising complex neuronal networks that regulate gastrointestinal motility, local blood flow, mucosal transport, and secretion. The gastrointestinal tract is colonized by a diverse and dynamically evolving population of microorganisms collectively known as the gut microbiome. These microorganisms play a crucial role in maintaining essential physiological processes, including digestion, nutrient absorption, metabolic regulation, and modulation of the immune response. In addition, they actively contribute to the maintenance of the intestinal barrier function and the regulation of local inflammatory processes.

Beyond its motor and secretory functions, the cellular components of the ENS are in close and dynamic communication with the gut microbiome. Contemporary studies emphasize the critical role of the gut-microbiome-brain axis in maintaining intestinal homeostasis, immune function, and overall neuropsychological well-being. A growing numbers of experimental and clinical evidence highlights the significant influence of the gut microbiome on the functioning of the gut-brain axis. Disrupted communication between the gut microbiome and the ENS is considered a key factor in the pathogenesis of gastrointestinal, neuropsychiatric, and neurodegenerative disorders. Understanding the mechanisms underlying this interaction is essential for preserving intestinal integrity and may provide new insights into the development of modern therapeutic strategies.

Keywords: enteric nervous system; microbiota; enteric glia; gut-microbiome-brain axis; neurological disorders;

ВЪВЕДЕНИЕ

Ентералната нервна система (ЕНС) е представена от множество неврони, нервни влакна и глиални клетки разположени в стената на храносмилателната система [1]. На фона на автономния контрол върху гастроинтестиналните функции, ЕНС е в тясно взаимодействие с чревния микробиом, който представлява комплексна и динамична общност от микроорганизми, която колонизира ГИТ. Двупосочната комуникация между микробиома и ЕНС излиза извън пределите на периферната нервна система (ПНС), включвайки централната нервна система (ЦНС), като по този начин допринася за интеграцията на процеси на ниво цял организъм.



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проф. Теодора Вълова, д.п.н.,
Председател на СУБ – Клон Плевен



Морфологични основи и дисфункция на ентералната нервна система при болестта на Хиршпрунг

Десислава Маринова¹, Любомира Ангелова², Стефан Трифонов³

Morphological basis and dysfunction of the enteric nervous system in Hirschsprung disease

Desislava Marinova¹, Lyubomira Angelova², Stefan Trifonov³

Abstract: The enteric nervous system (ENS) is a specialized autonomic neural network located within the wall of the gastrointestinal tract (GIT), which exerts independent control over intestinal motility, secretion, and local blood flow. It is organized into two main ganglionated plexuses – the myenteric plexus (Auerbach) and the submucosal plexus (Meissner). The myenteric plexus is situated between the circular and longitudinal layers of the muscularis externa, whereas the submucosal plexus is located within the submucosa, between the mucosa and the circular muscle layer. Through the coordinated activity of motor neurons, interneurons, primary sensory neurons, and glial cells, the ENS forms local reflex circuits that ensure normal peristalsis and intestinal transit. Neurons and glial cells of the ENS are derived from the neural crest. Impaired migration, proliferation, and differentiation of neural crest cells are associated with Hirschsprung disease (aganglionic megacolon). These disturbances in the embryonic development of the ENS lead to aganglionosis of the enteric plexuses, predominantly affecting the distal segments of the colon. All types of enteric neurons are absent, including excitatory cholinergic and inhibitory nitroergic (NO-producing) neurons, resulting in impaired relaxation of the smooth muscle, persistent contraction, and functional obstruction. Proximal to the affected segment, compensatory dilation and hypertrophy of the intestinal wall develop (megacolon).

This report provides an overview of the morphological characteristics of the ENS, as well as current diagnostic approaches and therapeutic management of Hirschsprung disease.

Keywords: enteric nervous system; neural crest; embryonic development; Hirschsprung disease; aganglionosis; intestinal motility disorders;

INTRODUCTION

The enteric nervous system (ENS), often referred to as the "second brain", has become a subject of sustained research attention, in part because of its capacity for autonomous function and the sheer scale of its neuronal population, estimated at several hundred million neurons distributed along the gastrointestinal tract [1, 2]. Although the ENS can generate intrinsic reflex activity independently, its function is still modulated by both sympathetic and parasympathetic inputs from the central nervous system [3]. Structurally, the ENS is organized within the gut wall into two major interconnected plexuses that differ both anatomically and functionally. The myenteric (Auerbach's) plexus, situated between the longitudinal and circular smooth muscle layers, is primarily involved in the regulation of gastrointestinal motility [1, 4]. In contrast, the submucosal (Meissner's) plexus, located within