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Review article

ACETYLCHOLINE AND ITS SYNTHESIZING ENZYME CHOLINE ACETYLTRANSPHERASE 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, mRNAsplicing 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. Nonganglionated 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-

cording to their morphological and functional properties, enteric neurons are classified into motor neurons, sensory neurons, and interneurons.

There are three types of motor neurons in the ENS: muscle motor neurons, secretomotor/visceromotor neurons and neurons that innervate entero-endocrine cells.

Muscle motor neurons innervate the longitudinal, circular muscles and muscular is mucosae through the digestive tract. Excitatory and inhibitory circular muscle motor neurons have a Dogiel type I shape. They have a stellar-shaped cell body with many short dendrites which divide several times into short, flat branches. Secretomotor and vasomotor neurons are two small classes of neurons which are more frequent in the submucosal plexus. Dogiel supposed secretomotor neurons to be type II neurons [3, 5, 6].

Sensory neurons along the ENS present a dense network of extrinsic sensory terminals and intrinsic primary sensory neurons. These two sensory systems are equally important for the sensory innervation of the gastrointestinal tract [10, 11]. Better known are the primary afferent terminals arising from sensory and parasympathetic ganglia of the vagus nerve (nodose ganglion and dorsal root ganglion). These fibers run predominantly in the vagal and spinal tracts. Because the cell bodies are located outside of the intestine, they are referred to as extrinsic primary afferent neurons (EPANs). The second type of sensory neurons are the intrinsic primary afferent neurons (IPANs). The bodies of these neurons are within the gut wall in the submucosal and myenteric plexus. The IPANs are multipolar cells with Dogiel II characteristics [12]. These sensory neurons supply most functional classes of enteric neurons. Whereas IPANs are essential for digestion control, EPANs notify the brain about processes that are relevant to homeostasis, pain, and the sensation of discomfort from the gastrointestinal tract.

Interneurons are ascending (orally directed) interneurons and several classes of descending (anally directed) neurons. Most of the ascending enteric interneurons belong to the Dogiel type I neurons. The majority of the inputs to the ascending interneurons come from IPANs, and the remaining are from other ascending interneurons, which form a chain of ascending excitation. They project orally within the myenteric plexus to synapse with the excitatory circular motor neurons. Most of the interneurons are from the descending type. In contrast to ascending ones, descending interneurons receive little input from IPANs, but rather from other descending interneurons. Descending interneurons are involved in local motility and secretomotor reflexes [3, 5, 6].

Neurochemical coding and electrical properties of the enteric neurons

The neurons along the enteric nervous system usually express a combination of different neurotransmitters. Co-expression of distinct transmitters depends on the type of neurons, the species, and the portion of the gastrointestinal tract. Acetylcholine, tachykinin, nitric oxide, adenosine triphosphate, vasoactive intestinal polypeptide [13, 14], calretinin and calbindin [15] are well-studied neurotransmitters in the ENS. Different types of synaptic events occur in the enteric circuits. These events include slow and fast excitatory postsynaptic potentials, inhibitory postsynaptic potentials and presynaptic inhibition and facilitation.

Excitatory motor neurons use acetylcholine, substance P and neurokinin A as neuromediators. There are two main types of intestinal secretomotor neurons – cholinergic and non-cholinergic. The ascending interneurons are mainly cholinergic, whereas the descending have a complex chemical coding, including acetylcholine, somatostatin, nitric oxide, and vasoactive intestinal polypeptide. Intrinsic primary afferent neurons use acetylcholine and tachykinin [3, 5, 6].

It is obvious that acetylcholine is the major excitatory transmitter in the enteric nervous system. It was discovered by Sir Henry Dale in 1914, and its existence was later confirmed by Otto Loewi.

Choline acetyltransferase, the synthesizing enzyme of acetylcholine

Acetylcholine, as a neurotransmitter, plays an important function in the peripheral nervous system as well as in the central nervous system. Its biosynthesis from acetyl-coenzyme A and choline is catalyzed by the enzyme choline acetyltransferase (acetyl-CoA, choline O-acetyltransferase, ChAT) in the cytoplasm of cholinergic neurons, and it is subsequently translocated into synaptic vesicles via the vesicular acetylcholine transporter (VAChT). In the central nervous system, acetylcholine plays a major role in many fundamental brain processes such as learning, memory, cognition, arousal, sleep and movement [16, 17], while in the spinal cholinergic motoneurons, it is responsible for the transmission of a nerve impulse at the neuromuscular junction. The cholinergic neurons are affected in neurodegenerative disorders like Huntington's disease [18], amyotrophic lateral sclerosis [19] and Alzheimer's disease [20], in which the expression level of ChAT mRNA per cholinergic cell was decreased [21] and selective loss of cholinergic neurons has been observed in the nucleus basalis magnocellularis [22]. Cholinergic neurons in the peripheral nervous system are involved in the control of visceral functions, and enteric cholinergic neurons play key roles in the control and regulation of gastrointestinal tract functions [23, 24]. Acetylcholine is also a non-neuronal signalling molecule in various cell types, in which ChAT expression has been demonstrated and may contribute to the regulation of a vast number of cell functions [25]. It has been suggested that non-neuronal acetylcholine can serve as a mediator for the development of inflammatory bowel disease and human colon cancer [26]. Morphological studies of the cholinergic system [27], together with genetic and molecular analysis of ChAT, as the most reliable marker of cholinergic structures [28], will provide the possibility to understand the physiologic and pathologic states of cholinergic neurons.

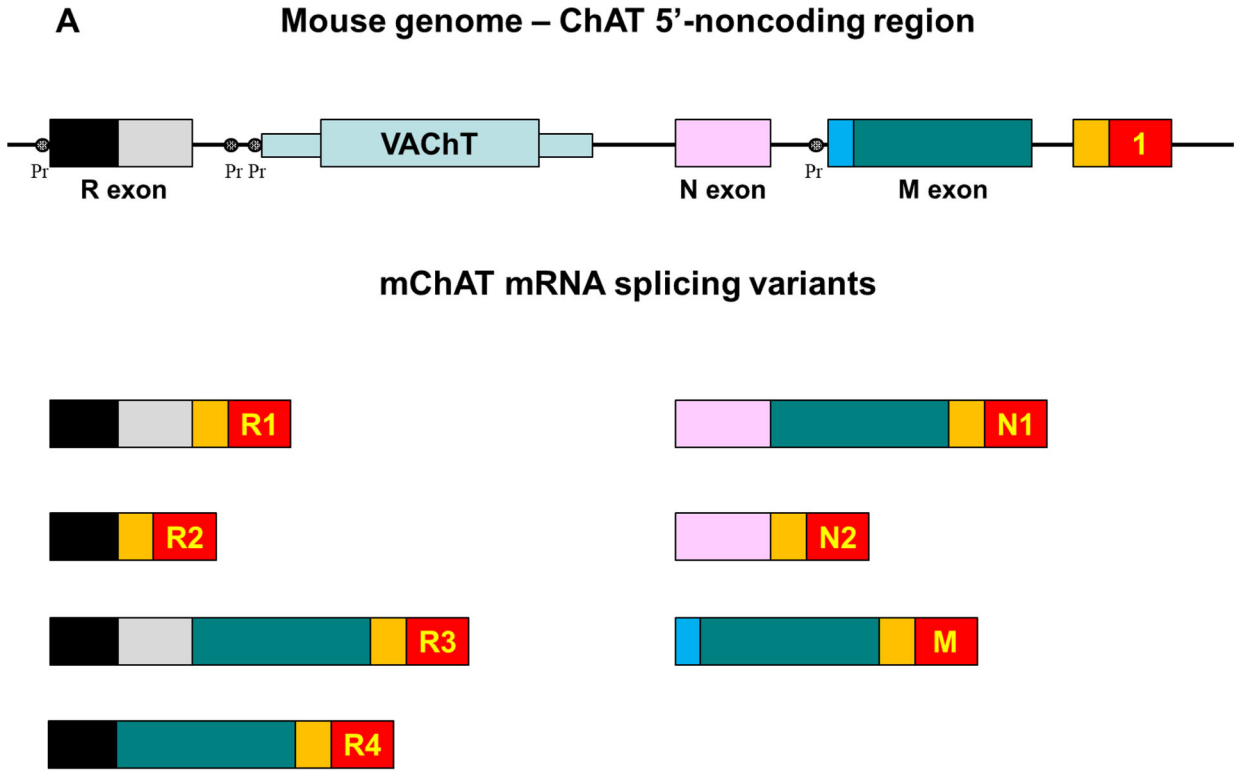
REVIEW RESULTS

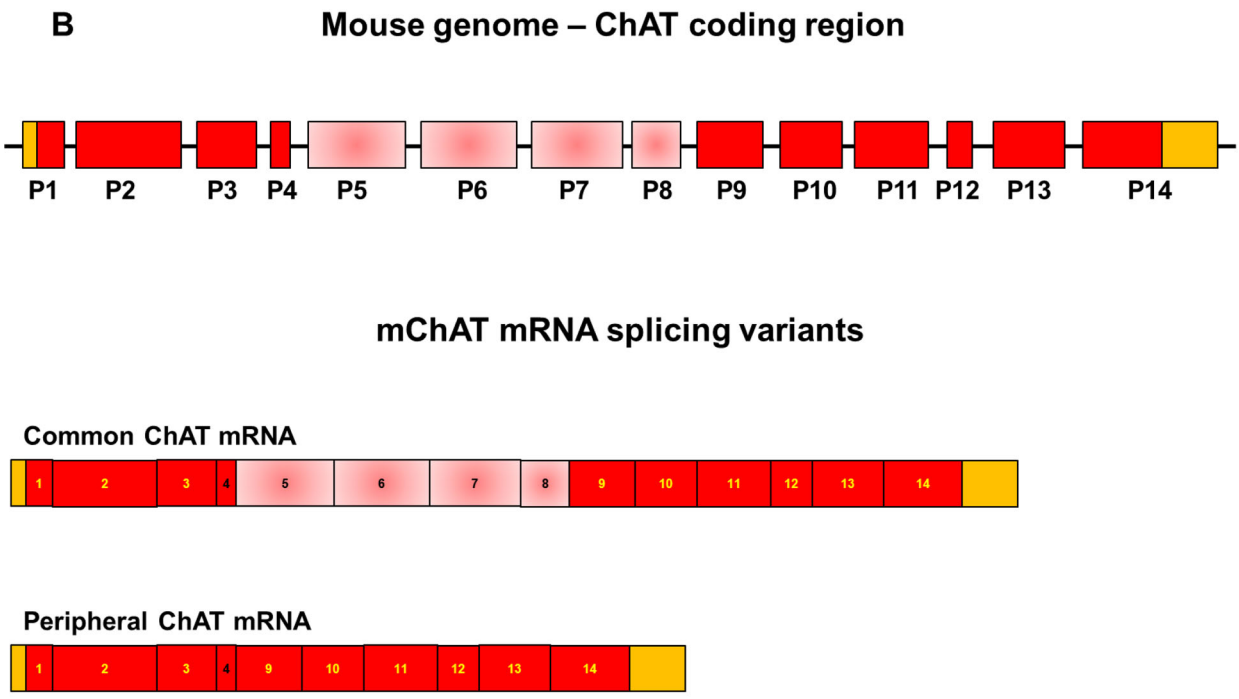
Structure of mouse cholinergic gene locus

The enzyme ChAT is encoded by a single gene in all species studied so far [29]. In the mouse, the gene encoding ChAT is composed of 17 exons. There are three 5'-noncoding exons (R, N and M), followed by 14 successive coding exons (Figure 1A, B) [30]. The entire open reading frame of VAChT mRNA is located in the first intron of the ChAT gene locus between R and N noncoding exons. Between the N exon and the first *N*-terminal coding, ChAT exon is located in the third M noncoding exon (Figure 1A). Seven differentially spliced mRNA isoforms (M, N1, N2, R1, R2, R3 and R4) are transcribed from the ChAT gene in mouse (fig. 1A.) [31], five variants in the rat (R1, R2, N1, N2, and M) [32] and six variants in human (R, N0, N1, N2, M and H) [33]. All these alternative splicing isoforms differ in their 5'-noncoding ends; therefore, all the transcripts encode the same protein, the 67-kDa common ChAT (cChAT). The functional role of these noncoding exons is not clear; probably, they impart different stabilities, translational efficiencies, compartmentalization and three-dimensional structures to their respective mRNA variants. The presence of several different promoter regions raises the possibility that different types of ChAT mRNA may be expressed in different types of cholinergic neurons and non-neuronal cells. Such a difference in the expression pattern of ChAT mRNA splice variants is reported in human leukemic T-cell lines. Major ChAT mRNA species in these cell lines were M-type and, to a lesser extent, N-type, but they did not express R-type ChAT mRNA [34]. In a previous *in situ* hybridization study of the distribution of these splicing variants in the CNS of the mouse, it was revealed that R1 and R2 were the dominant splice variants in the cholinergic neurons of forebrain nuclei, while R1, R2, R3, R4 and N1 splice variants were almost equally expressed in the motor and autonomic nuclei of the brainstem and the ventral and lateral horns of the spinal cord [35].

The presence of a splice variant of ChAT mRNA that lacks a serial sequence corresponding to coding exons from 5 to 8 was described in the rat (figure 1B) [36, 37]. Since the number of deleted nucleotides is an exact multiple of 3, the alternative splicing preserves the reading frame. The final product of expression of the alternative spliced mRNA is a smaller protein than cChAT, with a molecular weight of 49-kDa. The protein encoded by this alternative splice variant of ChAT mRNA is localized preferentially in peripheral nerve cells and fibers. Because of the dominant distribution in peripheral tissues, the novel variant was termed ChAT of a peripheral type (pChAT), and the full-length one was called ChAT of a common type (cChAT). The expression of cChAT and pChAT types is found in a variety of non-neuronal tissues and organs. In particular, the human and rat placenta [38] and human and murine immune cells [39] are well known to contain large amounts of acetylcholine and its synthesizing enzyme ChAT. pChAT is also widely expressed in the enteric nervous system of guinea pigs, sheep and rats [40].

Fig. 1. Schematic diagram showing the structure of cholinergic gene locus and the splicing pattern of multiple ChAT mRNA species. **A)** 5'-noncoding region and isoforms produced by alternative splicing of R, N and M noncoding exons. **B)** Coding region and the two alternatively spliced variants – cChAT and pChAT. Pr, promotor region; cChAT, common choline acetyltransferase; pChAT, peripheral choline acetyltransferase.





CONCLUSION

Even though the structure and regulatory elements of the mouse ChAT gene have received close attention in the past decades, knowledge of the distinct tissue-specific expression profiles of the ChAT splicing isoforms and the activity of the different promoters in mouse brain regions and non-neuronal tissues have remained incomplete. So far, only some ChAT mRNA splice variants have been discriminated by *in situ* hybridization, reverse transcription PCR and immunohistochemistry. These methods are only qualitative or semi-quantitative, and a truly quantitative and reproducible evaluation of ChAT mRNA splicing isoforms expression is still needed.

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