Acetylcholine synthesis by choline acetyltransferase of a peripheral type as demonstrated in adult rat dorsal root ganglion

氏　　名	BELLIER Jean-Pierre Roland
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審査委員	主査　教授　岡村富夫 副査　教授　工藤　基 副査　教授　陣内皓之祐

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論 文 内 容 要 旨

※整理番号	350	（ふりがな） 氏　名	ベリエ　ジャンピエール　ロラン BELLIER Jean-Pierre Roland
学位論文題目	Acetylcholine synthesis by choline acetyltransferase of a peripheral type as demonstrated in adult rat dorsal root ganglion. 「成熟ラット後根神経節における末梢型コリンアセチル基転移酵素によるアセチルコリン合成能の証明」		

Background

Choline acetyltransferase (ChAT), the synthesizing enzyme of acetylcholine (Ach), is the most reliable marker for cholinergic neurons. Our laboratory discovered a new isoform of ChAT and denominated it pChAT because of its preferential expression in the peripheral nervous system; the conventional enzyme was designated as cChAT. Immunohistochemistry using pChAT antiserum successfully visualized many known peripheral cholinergic cells that have never been labeled by cChAT antibodies. However, little is known about roles of pChAT, including its ability to synthesize Ach. To answer this question we used the adult rat dorsal root ganglion (DRG) neurons which have been immunohistochemically labeled by pChAT, but not cChAT. We examined whether pChAT can synthesize Ach in the ganglionic neurons.

Methods

Immunohistochemistry for pChAT and cChAT, using the avidin-biotin peroxidase procedure, was achieved on DRG, ventral and dorsal root of adult Wistar rat that had been intracardiacally perfused with fixative. Immunoblotting was completed after electrophoresis on SDS-PAGE. Laser capture microdissection was performed on 5 μm thick sections of the DRG stained with cresyl violet on a PixCell IIe LCM system. cDNA of the total RNA isolated from the whole DRG was obtained by reverse transcription using poly-dT12-18 oligonucleotides. cDNA from microdissected tissue was obtained using the cells-to-DNA II kit. ChAT cDNA of each sample was analyzed by PCR using a suitable set of primers. ChAT activity was assessed using a radiometric assay to detect Ach formed. A photometric assay using acetylthiocholine as substrate combined with specific cholinesterase inhibitors was used to assess specific acetyl- and butyryl-cholinesterases (ChEs) activities. Ach contents were measured by a chemiluminescent method. Some rats received a ventral root rhizotomy or dorsal root ligation at the level of the fourth lumbar segment. Immunoprecipitation under native conditions was performed using covalently bound pChAT antibody to a protein A

（備考）1. 論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、2千字程度でタイプ等で印字すること。

2. ※印の欄には記入しないこと。

matrix. The material bound on immunoaffinity matrix was directly used to estimate ChAT activity. Subsequently immunoprecipitated protein was eluted and used for western blotting or radiolabeled with [^{125}I]Iodine before electrophoresis on SDS-PAGE.

Results

pChAT antiserum labeled many small and partly medium to large-sized DRG neurons and numbers of axons of the dorsal root. cChAT immunolabelling was not detected in DRG. Western blot analysis revealed a single band for pChAT in DRG but not in the ventral root, and a single band for cChAT in ventral root but not in the DRG. mRNA expression analysis of laser microdissected cells indicated that neurons of any size express pChAT mRNA, but never cChAT mRNA. Significant values of ChAT activity, ChEs activity and Ach were detected in the crude extract of DRG and dorsal root of intact animals; those values were not affected by ventral root rhizotomy, indicating no contaminations by ventral root axons that contain high ChAT activity. An immunoprecipitated complex, consisting of tissue antigen trapped by pChAT antibody immobilized on protein A matrix, allowed us to detect a significant ChAT activity derived from DRG. After elution and radiolabeling, the immunoprecipitated displayed, on SDS-PAGE, a single band matching the size of the pChAT protein. Western blotting confirmed pChAT immunoreactivity of this band. Immunohistochemical and biochemical investigation of ligated dorsal root showed that both pChAT protein and ChAT activity became undetectable in the ligated root distal to the ganglion.

Discussion

Our immunohistochemical, western blotting and mRNA analyze indicate that pChAT is the only isoform of ChAT expressed in the DRG neurons. The formation of Ach was detectable in the DRG and dorsal root. Despite low enzyme activities of both ChAT and ChEs, the level of Ach in the ganglion was as high as to that in various brain regions receiving cholinergic innervation associated to the observation, suggesting that a stoichiometric balance between pChAT and AChE may regulate ACh contents in primary sensory afferents. DRG extract purified by a pChAT antibody-linked immunoaffinity column resulted in a single homogenous protein species exhibiting ChAT activity, indicating that pChAT possess a ChAT activity. Moreover, ligation of the dorsal root suggested that pChAT with ChAT activity is axonally transported from DRG toward the spinal cord.

Conclusions

We provide evidence that pChAT has definitely a ChAT enzymatic activity. We also show that pChAT is the only isoform of ChAT expresses in the DRG neurons, and that Ach is synthesized in the DRG. Then we conclude that pChAT is responsible for the production of ACh in primary sensory neurons of the DRG.

学位論文審査の結果の要旨

整理番号	３５０	氏名	BELLIER Jean-Pierre Roland

（学位論文審査の結果の要旨）

本研究は、蛋白としては未精製の末梢型アセチルコリン合成酵素について、独自の抗体を用いて、分子生物学的ならびに組織学的にその役割を検討したものである。

成熟ラット後根神経節に存在するアセチルコリン合成酵素（ChAT）は、中枢神経や運動神経に存在する中枢型（cChAT）ではなく、末梢型（pChAT）のみであることを明らかにした。また、同組織においてChAT活性、アセチルコリン含量、コリンエステラーゼ活性を測定し、前根側の神経に障害を与えてもそれらには影響がないことを観察した。さらに後根神経節の抽出物から得たpChATの免疫沈降物にChAT活性があること、後根を結紮することにより脊髄側の神経において、pChAT蛋白とその活性が消失することを見いだした。

本論文は、後根神経節におけるpChAT由来のアセチルコリン産生系の存在を明らかにし、知覚神経系におけるアセチルコリンの機能的役割を示唆するものである。これらは神経科学の発展に寄与するものであり、博士（医学）の学位を授与するに値すると評価された。

（平成　19年　8月　30日）