Neuroscience 234 (2013) 53–68

BUTYRYLCHOLINESTERASE AND THE CHOLINERGIC SYSTEM

G. A. REID,[a] N. CHILUKURI[b] AND S. DARVESH[a,c]*

[a] *Department of Medical Neuroscience Dalhousie University, Halifax, Nova Scotia, Canada*

[b] *Research Division, Physiology & Immunology Branch, US Army Medical Research, Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010, USA*

[c] *Department of Medicine (Neurology and Geriatric Medicine), Dalhousie University, Halifax, Nova Scotia, Canada*

Abstract—The cholinergic system plays important roles in neurotransmission in both the peripheral and central nervous systems. The cholinergic neurotransmitter acetylcholine is synthesized by choline acetyltransferase (ChAT) and its action terminated by acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The predominance of AChE has focused much attention on understanding the relationship of this enzyme to ChAT-positive cholinergic neurons. However, there is ample evidence that BuChE also plays an important role in cholinergic regulation. To elucidate the relationship of BuChE to neural elements that are producing acetylcholine, the distribution of this enzyme was compared to that of ChAT in the mouse CNS. Brain tissues from 129S1/SvImJ mice were stained for BuChE and ChAT using histochemical, immunohistochemical and immunofluorescent techniques. Both BuChE and ChAT were found in neural elements throughout the CNS. BuChE staining with histochemistry and immunohistochemistry produced the same distribution of labeling throughout the brain and spinal cord. Immunofluorescent double labeling demonstrated that many nuclei in the medulla oblongata, as well as regions of the spinal cord, had neurons that contained both BuChE and ChAT. BuChE-positive neurons without ChAT were found in close proximity with ChAT-positive neuropil in areas such as the thalamus and amygdala. BuChE-positive neuropil was also found closely associated with ChAT-positive neurons, particularly in tegmental nuclei of the pons. These observations provide further neuroanatomical evidence of a role for BuChE in the regulation of acetylcholine levels in the CNS.

Key words: cholinesterases, acetylcholinesterase, pseudocholinesterase, choline acetyltransferase, 129S1/SvImJ mice, acetylcholine.

*Correspondence to: S. Darvesh, Department of Medical Neuroscience, Dalhousie University, Room 1308, Camp Hill Veterans' Memorial, 5955 Veterans' Memorial Lane, Halifax, Nova Scotia, Canada B3H 2E1. Tel: +1-902-473-2490; fax: +1-902-473-7133.
E-mail address: sultan.darvesh@dal.ca (S. Darvesh).

Abbreviations: AChE, acetylcholinesterase; Ad-moBuChE, adenovirus containing the gene for mouse butyrylcholinesterase; Amb, ambiguus nucleus; AA, anterior amygdaloid area; ac, anterior commissure; ACo, anterior cortical amygdala; AD, anterior dorsal thalamus; AO, anterior olfactory cortex; AHP, anterior posterior hypothalamic nucleus; APT, anterior pretectal nucleus; ATg, anterior tegmental nucleus; AV, anterior ventral thalamus; Au, auditory cortex; BL, basolateral amygdala; BM, basomedial amygdala; BST, bed nucleus of stria terminalis; BSA, bovine serum albumin; BuChE, butyrylcholinesterase; CPu, caudate putamen nucleus; Ce, central amygdala; cp, cerebral peduncle; ChAT, choline acetyltransferase; Cg, cingulate cortex; Cl, claustrum; cc, corpus collosum; DpMe, deep mesencephalic nucleus; DAB, 3,3′-diaminobenzidine tetrahydrochloride; DLG, dorsal lateral geniculate nucleus; 10N, dorsal motor nucleus of the vagus; DR, dorsal raphe nucleus; DTg, dorsal tegmental nucleus; ELISA, enzyme-linked immunosorbent assay; En, endopiriform cortex; ec, external capsule; ECu, external cuneate; eml, external medullary lamina; 7N, facial nucleus; fr, fasciculus retroflexus; FBS, fetal bovine serum; fi, fimbria; f, fornix; g7, genu of the facial nucleus; Gi, gigantocellular reticular nucleus; GP, globus pallidus; H, hippocampal formation; HC, histochemical; HDB, horizontal limb nuclei of the diagonal band of Broca; hBuChE, human butyrylcholinesterase; 12N, hypoglossal nucleus; IgG, immunoglobulin G; icp, inferior cerebellar peduncle; IC, inferior colliculus; IO, inferior olivary nucleus; IS, inferior salivatory nucleus; I, insular cortex; InG, intermediate gray layer of superior colliculus; IRt, intermediate reticular nucleus; InWh, intermediate white layer of the superior colliculus; ic, internal capsule; IP, interpeduncular nucleus; InC, interstitial nucleus of Cajal; ICj, islands of Calleja; La, lateral amygdala; LDTg, lateral dorsal tegmental nucleus; LD, lateral dorsal thalamus; LEnt, lateral entorhinal cortex; LH, lateral hypothalamus; LPGi, lateral paragigantocellular nucleus; LP, lateral posterior thalamus; LRt, lateral reticular nucleus; LS, lateral septal nucleus; LVe, lateral vestibular nucleus; LC, locus coeruleus; MCPO, magnocellular preoptic nucleus; mt, mammillothalamic tract; MeA, medial anterior amygdala; MD, medial dorsal thalamus; MEnt, medial entorhinal cortex; MG, medial geniculate nucleus; MHb, medial habenula; ml, medial lemniscus; mlf, medial longitudinal fasciculus; MS, medial septal nucleus; MT, medial terminal nucleus of the accessory tract; MVe, medial vestibular nucleus; MnR, median raphe nucleus; MiTg, microcellular tegmental nucleus; M, motor cortex; Mo5, motor trigeminal nucleus; moBuChE, mouse butyrylcholinesterase; ns, nigrostriatal bundle; A5, noradrenaline cell group A5; LL, nucleus of lateral lemniscus; Tu, olfactory tubercle; opt, optic tract; Pa, paraventricular hypothalamic nucleus; PPTg, pedunculopontine tegmental nucleus; PAG, periaqueductal gray; PB, phosphate buffer; PBS, phosphate-buffered saline; Pir, piriform cortex; PoDG, polymorphic cell layer of the dentate gyrus; Pn, pontine nuclei; PnO, pontine reticular nucleus, oral part; pc, posterior commissure; PH, posterior hypothalamic nucleus; PMCo, posteromedial cortical amygdala; PMD, premammillary nucleus, dorsal; PMV, premammillary nucleus, ventral; PO, preoptic nucleus; Pr, prepositus nucleus; Pr5, principal sensory trigeminal nucleus; Po, pulvinar thalamic nucleus; py, pyramidal tract; rmoBuChE, recombinant mouse BuChE; R, red nucleus; Rt, reticular nucleus; RtTg, reticulotegmental nucleus of the pons; RS, retrosplenial cortex; Re, reuniens; RI, rostral interstitial nucleus of mlf; RPO, rostral paraolivary nucleus; rs, rubral spinal tract; S, sensory cortex; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; sol, solitary tract; Sol, solitary tract nucleus; Sp5I, spinal trigeminal nucleus, interpolaris; Sp5OVL, spinal trigeminal nucleus, ventrolateral oralis; sp5, spinal trigeminal tract; SVe, spinal vestibular nucleus; Or, stratum oriens; sm, stria medullaris; st, stria terminalis; SubC, subcoeruleus nucleus; SMT, submammillothalamic nucleus; SI, substantia innominata; SN, substantia nigra; scp, superior cerebellar peduncle; str, superior thalamic radiation; SuVe, superior vestibular nucleus; SuMM, supramammillary, medial nucleus; ts, tectospinal tract; TeA, temporal association cortex; TMB, 3,3′,5,5′-tetramethylbenzidine; m5, trigeminal nerve root; TBS, tris-buffered saline; VA, ventral anterior thalamic nucleus; VLG, ventral lateral geniculate; VP, ventral pallidum; VTA, ventral tegmental area; VDB, vertical limb nuclei of the diagonal band of Broca; V, visual cortex; ZI, zona incerta.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.12.054

INTRODUCTION

Cholinergic neurotransmission in the mammalian CNS is regulated predominantly by the enzyme acetylcholinesterase (AChE, EC 3.1.1.7) by catalyzing the hydrolysis of the cholinergic neurotransmitter acetylcholine (Silver, 1974). Improved histochemical techniques (Koelle and Friedenwald, 1949) for detecting this enzyme led to the first map of the distribution of AChE in the rodent brain (Shute and Lewis, 1963). Subsequently, antibodies were developed to detect choline acetyltransferase (ChAT, EC 2.3.1.6), the enzyme that catalyzes the synthesis of acetylcholine (Eng et al., 1974). This permitted elucidating the organization of cholinergic neurons (Kimura et al., 1980; Armstrong et al., 1983). Combined AChE histochemical and ChAT immunohistochemical staining studies demonstrated that most ChAT-positive neurons were also AChE-positive. (Eckenstein and Sofroniew, 1983; Levey et al., 1983, 1984).

In addition to AChE, the enzyme butyrylcholinesterase (BuChE, EC 3.1.1.8) is important in the regulation of the cholinergic system (Darvesh et al., 1998, 2003; Mesulam et al., 2002b; Giacobini, 2003; Duysen et al., 2007). Like AChE, BuChE is able to efficiently catalyze the hydrolysis of acetylcholine (Silver, 1974). BuChE is expressed in distinct populations of neurons, some of which also contain AChE (Friede, 1967; Tago et al., 1992; Darvesh et al., 1998; Darvesh and Hopkins, 2003; Geula and Nagykery, 2007). The importance of BuChE in cholinergic neurotransmission is further supported by the observation that AChE-knockout mice survive to adulthood. This indicates BuChE is able to compensate for the lack of AChE, allowing the continued regulation of cholinergic neurotransmission (Li et al., 2000; Xie et al., 2000; Mesulam et al., 2002a).

To date, study of the colocalization of BuChE and ChAT in the mammalian CNS has been limited to the spinal cord in the rat (Mis, 2005). Because of the earlier indications of BuChE co-regulation of cholinergic neurotransmission (Xie et al., 2000; Mesulam et al., 2002a,b), the present work was undertaken to examine the organization of BuChE-expressing neural elements as they relate to the ChAT-defined cholinergic system in the mouse CNS.

This work has not been presented elsewhere except in abstract form (Darvesh et al., 2012b).

EXPERIMENTAL PROCEDURES

Animals

Twenty male, wild-type (129S1/SvImJ) mice were purchased from The Jackson Laboratory (USA). This mouse strain was chosen because it has been utilized to examine components of the cholinergic system in other studies (Li et al., 2000; Mesulam et al., 2002a; Duysen et al., 2007). Animals were cared for according to the guidelines set by the Canadian Council on Animal Care. Formal approval to conduct the experiments was obtained from the Dalhousie University Committee on Laboratory Animals.

Materials

Unless otherwise stated, all reagents were purchased from Sigma–Aldrich (St. Louis, MO).

Preparation of brain tissue

Mice (between 10 and 18 weeks old) were deeply anesthetized with an intra-peritoneal injection of sodium pentobarbital (200 mg/kg) and perfused with approximately 25 ml of 0.9% saline solution containing 0.1% sodium nitrite followed by 50 ml of 0.1 M phosphate buffer (PB, pH 7.4) containing 4% paraformaldehyde. Brains were removed and post-fixed in PB with 4% paraformaldehyde for 1–2 h, cryoprotected and stored in PB with 30% sucrose and 0.05% sodium azide. Brains were cut in 40-μm serial sections in a coronal plane on a Leica SM2000R microtome with Physitemp freezing stage and BFS-30TC controller. Sections were stained for BuChE or AChE by histochemical (HC) technique, and for BuChE and ChAT using immunohistochemical (IHC) methods. Double labeling for BuChE and ChAT was performed using immunofluorescence (IF).

Cholinesterase histochemistry

Cholinesterase histochemical staining was performed using a modified (Darvesh et al., 2012a) Karnovsky–Roots method (Karnovsky and Roots, 1964). Briefly, tissue sections were rinsed in 0.1 M maleate buffer (pH 7.4) for 30 min and incubated for 1 h 45 min in 0.1 M maleate buffer (pH 8.0) containing 0.5 mM sodium citrate, 0.47 mM cupric sulfate, 0.05 mM potassium ferricyanide, 0.8 mM butyrylthiocholine iodide and 0.01 mM BW 284 C 51 (to inhibit AChE). All sections were then rinsed with gentle agitation for 30 min in dH_2O and placed in 0.1% cobalt chloride in water for 10 min. After further rinsing in dH_2O, sections were placed in PB containing 1.39 mM 3,3′-diaminobenzidine tetrahydrochloride (DAB). After 5 min in the DAB solution, 50 μl of 0.15% H_2O_2 in dH_2O was added per ml of DAB solution, and the reaction was carried out for approximately 3 min. Sections were then washed in 0.01 M acetate buffer (pH 3.3), mounted on slides, cleared in xylene and cover-slipped. Control experiments to demonstrate specificity of BuChE staining were performed as described previously (Darvesh et al., 1998) and indicated the staining pattern observed was specific for BuChE activity.

The procedure for the visualization of AChE activity was similar to that for BuChE except that the reaction solution was 24.99 mM sodium citrate, 14.72 mM cupric sulfate, 2.43 mM potassium ferricyanide, 2.46 mM acetylthiocholine iodide and 0.13 mM ethopropazine (to inhibit BuChE), at room temperature in 0.1 M maleate buffer (pH 6.0) for 15 min with gentle agitation.

Generation and characterization of polyclonal antibodies to BuChE

Rabbit polyclonal antibodies to recombinant mouse BuChE (rmoBuChE) were generated for this study. Production of rmoBuChE (immunogen) was accomplished in human embryonic kidney epithelial cells (293A cells) using an adenovirus containing the gene for mouse BuChE (Ad-moBuChE) (Parikh et al., 2011). The mouse BuChE (moBuChE) gene contained a 6x histidine tag at its carboxyl terminus suggesting that the rmoBuChE used was a fusion protein. Large-scale expression of rmoBuChE was accomplished by overnight culturing of 293A cells (10×10^6) in 150-cm^2 tissue culture dishes and infecting them for 1 h with 10 μl of 4th cycle crude viral lysate (high titer CVL) of Ad-moBuChE in 10 ml of infection medium (DMEM containing 2% fetal bovine serum (FBS), antibiotics penicillin and streptomycin