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Cellular protein and mRNA expression of β1 nicotinic acetylcholine receptor (nAChR) subunit in brain, skeletal muscle and placenta

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ABSTRACT

The β1 nicotinic acetylcholine receptor (nAChR) subunit is a muscle type subunit of this family and as such, is found predominantly in muscle. Recent reports document its expression in other tissues and cell lines including adrenal glands, carcinomas, lung and brain. However, the majority of studies were of tissue lysates, thus the cellular distribution was not determined. This study aimed to determine the cellular distribution of the β1 nAChR subunit in the brain, at both the mRNA and protein levels, using non-radioactive *in situ* hybridization (ISH) and immunohistochemistry (IHC), respectively, and to compare it to two muscle tissue types, skeletal and placenta. Tissue was formalin fixed and paraffin embedded (all tissue types) and frozen (placenta) from humans. Additional control tissue from the piglet and mouse brain were also studied, as was mRNA for the α3 nAChR and N-methyl-D-aspartate receptor 1 (NR1) subunit. We found no β1 nAChR subunit mRNA expression in the human and piglet brain despite strong protein expression. Some signal was seen in the mouse brain but considered inconclusive given the probes designed were not of 100% homology to the mouse. In the skeletal muscle and placenta tissues, β1 nAChR subunit mRNA expression was prominent and mirrored protein expression. No α3 nAChR or NR1 mRNA was seen in the skeletal muscle, as expected, although both subunit mRNAs were present in the placenta. This study concludes that further experiments are required to conclusively state that the β1 nAChR subunit is expressed in the human, piglet and mouse brain.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are part of the Cys-loop family of ligand gated ion channels. The receptor is formed as a pentamer, with five subunits arranged around a central pore that is permeable to cations upon binding of an agonist, such as acetylcholine (ACh) or nicotine. A total of seventeen nAChR subunits have been characterized and two types of nAChRs are known; muscle type and neuronal type. The β1 nAChR subunit is traditionally characterized as a muscle type subunit, expressed in either $(\alpha1)_2\beta1\gamma d$ or $(\alpha1)_2\beta1\varepsilon d$ receptor complexes (Millar, 2003; Mishina et al., 1985). Receptors containing β1 subunits have been found predominantly in the neuromuscular junction (NMJ), and have been attributed to the efficient clustering of nAChRs and anchoring of the receptors to the cytoskeleton (Wheeler et al., 1994). This is important for formation of synapses in the NMJ, evidenced by data where the exchange of the β1 with β2 subunits in nAChRs expressed in *Xenopus* oocytes resulted in decreased cluster formation of the receptor proteins (Kalamida et al., 2007; Wheeler et al., 1994). Furthermore, lack of the β1 subunit in $\alpha1\gamma\delta$ complexes resulted in weaker channel activity upon agonist binding (Kalamida et al., 2007).

The characterization of nAChRs in skeletal muscle of rat has been extensively reviewed (Schuetze and Role, 1987), with more recent data reporting expression of the β1 nAChR subunit mRNA (GTEx; www.gtexportal.org/home/gene/CHRNB1) and protein within muscle tissue as well as other rat and human peripheral tissue types including liver, adrenal medulla, bronchial cells, and the placenta, summarized in Table 1. However, most of these studies applied polymerase chain reaction methods for mRNA expression and immunoblotting for protein expression; thus, the cellular distribution of the subunit has not been delineated. Expression at the cellular level provides novel data in determining cell type-specific functionality. *In situ* hybridization (ISH) and immunohistochemistry (IHC) are two common techniques used in cellular

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Table 1
Summary of studies of β1 nAChR subunit mRNA (CHRNB1) and protein in peripheral tissues by chronological order.

Tissue/Cell line type	Species	Technique	References
Soleus skeletal muscle	Rat	RNase protection assay, ISH	(Goldman and Staple, 1989)
Mononucleated myogenic cell culture	Mouse	RNase Protection Assay	(Martinou et al., 1991)
Soleus skeletal muscle	Rat	ISH	(Kues et al., 1995)
Adipocytes	Rat	RT-PCR	(Liu et al., 2004)
Bronchial epithelial cell culture, airway fibroblasts	Human	RT-PCR WB	(Carlisle et al., 2004)
Cervix squamous CaSki and SiHa cell lines and adenocarcinoma HeLa cells	Human	RT-PCR IHC	(Calleja-Macias et al., 2009)
L6 Myoblast cell lines ATCC-PC12 cells HepG2 cancer cell line	RatHuman	qRT-PCR	(Wadensweiler, 2012)
All tissue organs	Human	Gene modelling/eQTL	http://www.gtexportal.org/home/gene/CHRNB1 (2013)
All tissue organs	Human	IHC	The Human Protein Atlas online
Maxilla	Rat	qPCR	(Guo et al., 2014)
Placenta	Human	qRT-PCR, WB	(Machaalani et al., 2015; Machaalani et al., 2014)

Abbreviations: RT-PCR; Reverse transcription polymerase chain reaction; qPCR; Real-time polymerase chain reaction; qRT-PCR; RT-PCR and qPCR combined; eQTL; Expression quantitative trait loci; WB; Western Blotting; ISH; *in situ* hybridization; IHC; Immunohistochemistry.

localization of mRNA and protein, respectively. To date, only two studies examining β1 subunit mRNA expression at the cellular level have been conducted, both in rat skeletal muscle (Goldman and Staple, 1989; Kues et al., 1995).

Investigations into the expression of the β1 nAChR subunit in the brain or brain-like structures are more recent, and are summarized in Table 2. A diversity of species has been examined, including cockroach, bee, human, mouse and piglet brain regions. These studies include two from our laboratory (Vivekanandarajah et al., 2016; Vivekanandarajah et al., 2015) using a rabbit polyclonal antibody (sc-11371), where we found β1 subunit protein expressed in piglet (Vivekanandarajah et al., 2015) and mouse (Vivekanandarajah et al., 2016) brainstem and hippocampus. We also showed that nicotine exposure led to a greater change in β1 subunit expression compared to the subunits characteristic of the neuronal type nAChRs that were simultaneously studied, including α2-7, α9, and β2 (Vivekanandarajah et al., 2015; Vivekanandarajah et al., 2016).

The current study was conducted to identify β1 subunit mRNA and protein expression in human, mouse and piglet brain, human skeletal muscle and human placenta. Mapping both mRNA and protein expression permitted determination of whether any observed changes in subunit protein expression were correlated with changes in mRNA levels. We used non-radioactive ISH which allowed for the cellular visualization of the mRNA, and IHC for cellular visualization of β1 subunit immunoreactive protein. Since no β1 subunit mRNA signal was detected in the brain of any species tested, we further report here on the methods applied to determine possible reasons for the non-homology between mRNA and protein expression of the β1 nAChR subunit at the cellular level in brain.

2. Method and materials

2.1. Formalin fixed and paraffin embedded (FFPE) tissue

Various types of tissues, both animal and human, were used in this study and were available in our laboratory under ethics protocols approved by the Human Research Ethics Committees of The University of Sydney and The Sydney Local Health District (Royal Prince Alfred Hospital Zone), and the Animal Ethics Committee of The University of Sydney. The human hippocampus, midbrain, brainstem and cortex (anterior superior frontal lobe) (obtained from the Department of Forensic Medicine, Glebe, NSW, Australia), were fixed in 20% neutral buffered formalin (NBF) for a period of 2–23 days and paraffin embedded using a 3-day processing protocol. Human placenta was collected within 30 mins of delivery, washed with phosphate buffered saline (PBS; 09-2051-100, Medicago, Sweden) and fixed in 10% NBF for 1 day, then paraffin embedded overnight (detailed in Machaalani et al., 2014). Adult

Table 2
Summary of studies of β1 nAChR subunit mRNA (CHRNB1) and protein in brain tissue.

Tissue/Cell line type	Species	Technique	Reference
Cultured Kenyon cells of mushroom bodies	Adult Bees	Whole cell-patch clamp, single-cell RT PCR	(Dupuis et al., 2011)
Mushroom bodies Kenyon cells	Cockroach	qRT-PCR	(Taillebois and Thany, 2016)
All brain regions	Human	Gene modelling/eQTL	http://www.gtexportal.org/home/gene/CHRNB1 (2013)
Pluripotent stem cell derived neurons	Human	qRT-PCR, Calcium fluorescence, FLIPR-bases intracellular calcium assay, Whole cell patch clamp	(Chatzidaki et al., 2015)
Cortex, hippocampus, cerebellum	Human	IHC	The Human Protein Atlas [Online]
Brainstem medulla, hippocampus	Piglet	IHC	(Vivekanandarajah et al., 2015)
Brainstem medulla, hippocampus	Mouse	IHC	(Vivekanandarajah et al., 2016)

Abbreviations: eQTL; Expression quantitative trait loci; RT-PCR; Reverse transcription polymerase chain reaction; qPCR; Real-time polymerase chain reaction; qRT-PCR; RT-PCR and qPCR combined; FLIPR; Fluorescent imaging plate reader; IHC; Immunohistochemistry.