Topic Introduction

The Synaptosome as a Model System for Studying Synaptic Physiology

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Alongside rodent brain slices and primary neuronal cultures, synaptosomes (isolated nerve terminals) have been an important model system for studying the molecular mechanisms of synaptic function in the brain. Synaptosomes were first prepared in the late 1950s by Whittaker and colleagues and were instrumental in studying synaptic structure and defining the functional components of the synapse, including the identity of the major neurotransmitters and their uptake mechanisms. Synaptosomes can also be stimulated to release neurotransmitters and were used to discover a number of regulatory signaling pathways that fine-tune synaptic transmission. In the past decade, landmark proteomic studies of synaptosomes and synaptic vesicle preparations have further dissected the protein composition of the synapse. This introduction briefly describes the history of the synaptosome preparation and highlights how it continues to be relevant as our focus in the neuroscience community centers on synaptic dysfunction in aging and neurological disease.

BACKGROUND

The neuronal chemical synapse is the fundamental site of communication between neurons, and for each of the approximately 86 billion neurons of the human brain there can be more than 10^5 synaptic contacts (Napper and Harvey 1988; Azevedo et al. 2009). A typical presynaptic nerve terminal of the central nervous system has approximately 100 to 200 neurotransmitter-filled synaptic vesicles (Fig. 1A) available to deposit their contents into the synaptic cleft in a Ca^{2+} -dependent manner upon action potential stimulation (Sudhof 2013). The released neurotransmitter diffuses across the synaptic cleft to interact with specific postsynaptic ionotropic and metabotropic receptors that mediate propagation of the action potential. Regulation of this process, the alteration of synaptic strength, is the molecular basis for learning and the storage of memories (reviewed by Mayford et al. 2012). Our knowledge of the protein components of synaptic transmission and the principles of their function are now well characterized (Sudhof 2013), but owe a great debt to the synaptosome preparation (isolated nerve terminals) for many key discoveries. To illustrate this point, a PubMed search of the term “synaptosome” yields more than 13,000 publications.

SYNAPTOSOMAL PREPARATIONS

The enrichment of synaptic fractions from homogenized brain tissue was first performed in the late 1950s (Hebb and Whittaker 1958) and validated in the 1960s, with the observation by electron microscopy that purified intact nerve endings or synaptosomes resembled synaptic structures in

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FIGURE 1. Preparation and composition of the synaptosome. (A) Schematic representation of a synapse, indicating where the presynaptic nerve terminal (upper dotted line) and attached postsynaptic density (lower dotted line) pinch off following homogenization of brain tissue to form a synaptosome. (B) Labels indicate synaptic vesicles (SV), mitochondria (mito), and the postsynaptic density (PSD). (C) An electron micrograph of a rat brain synaptosome, illustrating the features in B. Scale bar, 100 nm. (Micrograph courtesy of Professor M.A. Cousin, University of Edinburgh.)

tissue (Gray and Whittaker 1962). The basis of all synaptosomal preparations is the initial homogenization of fresh brain tissue with a glass–Teflon homogenizer, generating shear forces that pinch off nerve terminals, which then reseal to form synaptosomes (Fig. 1A,B). The PSD often remains attached to the synaptosome, presumably because of the trans-synaptic protein complexes that physically link the two compartments (Fig. 1C; Gray and Whittaker 1962). A crude enrichment of synaptosomes can be achieved by centrifuging the homogenate at low speed to pellet myelin and other debris and then centrifuging the resulting supernatant at high speed to yield a microsomal P2 pellet. A purer preparation can be obtained by applying the crude synaptosomes to a Percoll gradient (Dunkley et al. 2008). Synaptosomes are amenable to both structural and functional studies of the synapse because they not only can provide sufficient material for protein biochemical experiments, but also can maintain metabolic activity and membrane potential and can be stimulated to release neurotransmitter. The synaptosome preparation also acts as a basis for further subcellular fractionation, including synaptic vesicles (Nagy et al. 1976; Huttner et al. 1983; Hell et al. 1988), the presynaptic cytomatrix, and the postsynaptic density (PSD) (Phillips et al. 2001).

In the accompanying protocol, simple centrifugation techniques are used to sequentially subfractionate rodent brain tissue and prepare both synaptosomes and synaptic vesicles (see Protocol: **Subcellular Fractionation of the Brain: Preparation of Synaptosomes and Synaptic Vesicles** [Evans 2014]). The resulting preparations are suitable for functional and protein biochemical studies of the synapse.

USES FOR SYNAPTOSOMAL PREPARATIONS

Synaptosome experiments were instrumental in first identifying neurotransmitters, including the proof that amino acids, such as glutamate, were indeed neurotransmitters. Typical approaches involved loading and detecting the evoked release of radioactively labeled neurotransmitters, including GABA, glutamate, acetylcholine, dopamine, and noradrenaline (Levy et al. 1973), or detecting endogenous release by high-performance liquid chromatography. Later developments led to the real-time detection of endogenous glutamate exocytosis via a fluorometric enzyme-linked assay (Nicholls et al. 1987) and synaptic vesicle endocytosis via the uptake of fluorescent styryl dyes (Marks and McMahon 1998). With the advent of pharmacological tools to manipulate ion channels and receptors, a host of experiments in synaptosomes helped define the major regulatory signaling pathways that regulate neurotransmission. For example, a large body of work has focused on how different classes of metabotropic glutamate autoreceptors regulate glutamate release via G-protein-mediated effects on phospholipases, kinases, and voltage-gated ion channels (Herrero et al. 1992; Rodriguez-Moreno et al. 1999).

In more recent years, synaptosomes prepared from knockout or knock-in mice have been used to support studies into the roles of synaptic proteins (e.g., Lonart et al. 1998; Pozzo-Miller et al. 1999; Lonart and Simsek-Duran 2006; Sumioka et al. 2011). Synaptosomes and synaptic vesicle preparations have also been subjected to proteomic and phosphoproteomic analysis (Collins et al. 2005; Schrimpf et al. 2005; Munton et al. 2006). In one landmark study, the protein and lipid components of the synaptic vesicle preparation were meticulously characterized, revealing more than 80 integral membrane proteins and more than 100 further peripheral proteins associated with the synaptic vesicle membrane (Takamori et al. 2006). These proteomic studies have, first, cataloged the incredible number of neuronal-specific proteins operating at the synapse, and, second, underlined the vast array of posttranslational modifications that these proteins are subjected to and how they change in response to synaptic activity. Dissecting the networks of synaptic protein–protein interactions and how these are regulated in health and disease is the current challenge in synaptic research and will no doubt continue to be supported by experimental approaches using the synaptosome preparation. Indeed, the most recent synaptosome literature is predominated by studies of genes that are mutated in disease and includes the preparation of synaptosomes from the postmortem brains of human patients (e.g., Liao et al. 2008; Sokolow et al. 2011; Harish et al. 2012; Kopeikina et al. 2012; Vitale et al. 2012).

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