Review
The glutamatergic nerve terminal

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Glutamate as a neurotransmitter

The human brain has about $10^{10}$ neurones, each of which, on average, makes about 1000 synaptic contacts with other neurones. Of these $10^{13}$ synapses perhaps up to 90% utilize amino acids as their neurotransmitter. Glutamate is the major excitatory amino acid (acting predominantly on depolarizing post-synaptic receptors) while 4-aminobutyrate (GABA) and glycine are the major inhibitory transmitters (acting on hyper-polarizing receptors). As well as playing the dominant role in fast information transfer, glutamate is of particular interest in view of its involvement in current models of memory and learning (Bliss and Dolphin, 1982; Cotman et al., 1988; Collingridge and Singer, 1990) and because a pathological release of glutamate in brain ischaemia is neurotoxic and a major contributor to the damage caused under these conditions (Rothman and Olney, 1986; Choi, 1988; Meldrum and Garthwaite, 1990).

A synapse has a pre-synaptic element responsible for the storage and release of transmitter and a post-synaptic element containing one or more classes of receptor for the transmitter. The potent combination of electrophysiology and molecular cloning has allowed a dramatic advance in our understanding of post-synaptic events. Three classes of post-synaptic ionotropic (possessing an integral ion channel) glutamate receptors have been identified and cloned: the 2-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA/KA) receptor (Hollmann et al., 1989; Nakamichi et al., 1990; Sakmann, 1992), the high-affinity KA receptor (Egebjerg et al., 1991) and the N-methyl d-aspartate (NMDA) receptor (Moriyoshi et al., 1991; Kumar et al., 1991; Barnard, 1992). Each can exist in many isoforms by combining subunits formed from distinct genes, by alternative splicing or by post-transcriptional modification (Monyer et al., 1991; Burnashev et al., 1992).

The AMPA/KA receptor is responsible for fast information transfer while the NMDA receptor is only operative when the post-synaptic membrane is independently depolarized by another receptor. This ‘associative’ behaviour of the latter is believed to underlie aspects of the learning process.

Unfortunately the conductance characteristics of the NMDA receptor (a high conductance to Ca$^{2+}$ and slow inactivation) may be responsible for much of the excitotoxicity of glutamate in ischemia. Since these ionotropic receptors have a post-synaptic location they will not be discussed further in this review.

In addition to the ionotropic glutamate receptors, a family of metabotropic glutamate receptors coupled to G-protein-binding protein (G-protein) has been identified (Sladecek et al., 1985; Sugiyama et al. 1987; Schoepp et al., 1990) and cloned (Masu et al., 1991; Tanabe et al., 1992). These may be either post- or pre-synaptic and may be either coupled positively to inositol phosphate turnover or negatively to adenylyl cyclase activity (Anwyl, 1991). A putative L(+)-2-amino-4-phosphonobutyrate receptor has now been identified with the type IV metabotropic glutamate receptor which is negatively coupled to adenylyl cyclase (Thomsen et al., 1992). G-protein-coupled receptors play a predominantly modulatory role in neurotransmission.

In parallel with these advances in understanding of the receptors, the presynaptic mechanisms governing the uptake and release of transmitter glutamate have been investigated. Two of the most fruitful approaches have been the ‘functional’, where the action of the intact, isolated nerve terminal, or synaptosome, has been dissected, and the ‘structural’, where the proteins which are uniquely expressed in the presynaptic terminal are identified and characterized. The latter has been the subject of a number of detailed recent reviews (Greengard, 1987; De Camilli and Jahn, 1990; De Camilli et al., 1990; Sudhof and Jahn, 1991) and this review will refer only briefly to such studies, instead concentrating upon studies with intact synaptosomes. Part of the current excitement in the field, however, is the realization that these ‘top-down’ and ‘bottom-up’ approaches are beginning to intermesh to provide an integrated view of presynaptic mechanisms.

Features of synaptosomal function which appear to be common to glutamatergic and non-glutamatergic terminals will be included in this review. However to keep the scope of the review within bounds, the only transmitter which will be discussed in any detail is glutamate itself.

The synaptosome

The synaptosomal preparation has been available since the 1960s for the investigation of presynaptic function (Gray and Whittaker, 1962). It is prepared by the careful homogenization of defined brain areas: the neck of the axon at the point at which it enters the nerve terminal appears to be par-
Fig. 1. The presynaptic terminal with the planes of cleavage (dotted arrows) during the synaptosomal preparation. Typical synaptosomes contain many small electron-lucid synaptic vesicles concentrated in the region of the active zone where exocytosis occurs. Occasional peptide-containing large dense-cored vesicles are seen located away from the active zones.

particularly fragile, perhaps due to changes in cytoskeletal organization, and readily pinches off leaving a resealed isolated intact nerve terminal (Fig. 1), or synaptosome which can be partially purified by gradient centrifugation (Booth and Clark, 1978; Dunkley et al., 1986). It should be emphasized that the in-situ nerve terminal functions largely autonomously from the exceedingly distant cell body, requiring only the electrical signal from the axonal action potential to trigger release and the replenishment of materials via axonal transport mechanisms for long-term survival. Synaptosomes can be maintained on ice for at least 6 h with no deterioration in their ability to maintain their ATP levels, ion gradients or ability to release neurotransmitter.

Synaptosomes have a characteristic appearance with a diameter of 0.5–1 μm, containing one or more small mitochondria and frequently with an area of post-synaptic membrane adhering to it opposite a concentration of 50-nm-diameter small electron-lucid synaptic vesicles. There is little or no internal membrane corresponding to endoplasmic reticulum.

The synaptosomal preparation has particular advantages and problems. It is the simplest preparation which retains all the machinery for the uptake, storage and release of transmitters. It is thus largely depleted of functional glial and neuronal cell body elements. Although electron micrographs of synaptosomal preparations sometimes look unpleasantly heterogeneous, the non-synaptosomal membrane fragments are generally ‘dead’ from a functional point of view, and do not interfere with studies which require an intact membrane, glycolytic pathway, mitochondria and ion gradients. One problem which remains, however, is that mammalian central nervous system (CNS) synaptosomes are inherently heterogeneous in terms of transmitter content, since even the most closely defined anatomical region contains a wide variety of transmitters. This problem is less severe for glutamate, which in terms of release is the dominant transmitter in most synaptosomal preparations, but it does mean for example that not all of a depolarization-evoked Ca²⁺ signal or change in phosphorylation state of a presynaptic protein can be assumed to be coupled to glutamatergic transmission, and it is important that this reservation is borne in mind in all synaptosomal studies which do not involve a transmitter-homogeneous preparation.

An overview of synaptosomal bioenergetics

Synaptosomes can utilize glucose both aerobically and anaerobically and pyruvate as an oxidative metabolite (Kauppinen and Nicholls, 1986). Despite the presence of high concentrations of glutamate and aspartate within the synaptosomal cytoplasm, these are not readily utilizable as energy sources (Kauppinen and Nicholls, 1986). There is a large excess capacity of glycolysis to maintain ATP in aerobic synaptosomes. Thus when ATP synthesis by the intrasynaptosomal mitochondrial is inhibited by anoxia and the terminal is forced to rely on anaerobic glycolysis to lactate, a 10-fold increase in glucose utilization can be observed and there is only a moderate fall in ATP levels (Kauppinen and Nicholls, 1986). Carefully prepared synaptosomes retain a high respiratory control, i.e. respiration can be stimulated 5–10-fold by the addition of mitochondrial uncouplers (Scott and Nicholls, 1980; Kauppinen and Nicholls, 1986). They maintain a plasma membrane potential of −60 mV to −80 mV in low K⁺ medium (Blaustein and Goldring, 1975; Scott and Nicholls, 1980; Agoston et al., 1983) together with a volume-average cytoplasmic free Ca²⁺ concentration, [Ca²⁺], of 0.1–0.2 μM (Ashley et al., 1984; Nachshen, 1985a; Verhage et al., 1988; Kauppinen et al., 1988). It may thus be concluded that the preparation is energetically ‘intact’.

Glutamate retrieval from the synaptic cleft

In common with other non-peptide transmitters, glutamate undergoes a cycle of retrieval from the synaptic cleft by a Na⁺-coupled co-transport pathway, accumulation into synaptic vesicles and exocytotic release. Two retrieval mechanisms have been investigated: direct reuptake into the terminal and reuptake into neighbouring glial cells followed by conversion to glutamine, transport of glutamine into the terminal and hydrolysis to glutamate. The relative contributions of the two pathways is a matter of debate.

The plasma membrane acidic amino acid transporter

The plasma membrane Na⁺-coupled cotransport pathway for the uptake of glutamate (Fig. 2) is responsible for removing glutamate from the synaptic cleft and maintaining an ex-