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Physiological separation of vesicle pools in low- and high-output nerve terminals

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ABSTRACT

Physiological differences in low- (tonic like) and high-output (phasic like) synapses match many of the expected anatomical features of these terminals. However, investigation in the recruitment of synaptic vesicles from a reserve pool (RP) to a readily releasable pool (RRP) of synaptic vesicles within these types of nerve terminals has not been fully addressed. This study highlights physiological differences and differential modulation of the vesicles in a RP for maintaining synaptic output during evoked depression of the RRP. With the use of bafilomycin A1, a vacuolar ATPase blocker, recycling vesicles are blocked in refilling with transmitter. The tonic terminal is fatigue resistant due to a large RRP, whereas the phasic depresses rapidly upon continuous stimulation. These differences in rates of depression appear to be in the size and degree of utilization of the RRP of vesicles. The working model is that upon depression of the tonic terminal, serotonin (5-HT) has a large RP to act on in order to recruit vesicles to the RRP; whereas, the phasic terminal, 5-HT can recruit RP vesicles to the RRP prior to synaptic depression but not after depression. The vesicle pools are physiologically differentiated between phasic and tonic output terminals.

1. Introduction

Chemical synaptic communication at neuromuscular junctions (NMJs) generally occurs by synaptic vesicles, packaged with a transmitter, fusing with the presynaptic plasma membrane to release the transmitter into a synaptic cleft for the postsynaptic receptors to receive and respond accordingly. How vesicles fuse with the presynaptic membrane is an active area of investigation on a comparative scale as it is assumed there are a variety of ways this process may occur from kiss-and-run to full exocytosis of vesicles (Rosenmund and Stevens, 1996; Aravanis et al., 2003; Rizzoli et al., 2003; Sudhof, 2004; Rizzoli and Betz, 2005; Fredj and Burrone, 2009). The recycling and repacking of the vesicles are also of substantial interest particularly given that there appear to be different pools of vesicles for various functions within presynaptic nerve terminals and unique processes as well as serving similar roles among various animal species (Atwood and Cooper, 1996a,b; Sudhof, 2004; Rizzoli and Betz, 2005; Denker et al., 2011a,b).

Recently it was suggested that synaptic vesicles may not just serve as a means of packaging transmitter but also providing essential proteins as a buffer source for use when needed (Denker et al., 2011a,b). In addition, a large influx of Ca^{2+} can depress synaptic transmission (Katz and Miledi, 1969; Heuser et al., 1971; Ohta and Kuba, 1980). Also, acidification within the nerve terminals depresses vesicles endocytosis (Lindgren et al., 1997). These processes may serve as potential negative feedback mechanisms. Such observations raise questions about the functional needs of reserve pool (RP) and readily releasable pool (RRP) of vesicles and their roles. To determine the functional differences, in terms of vesicle recycling and recruitment in the RP and the RRP between phasic (high-output) and tonic (low-output) motor nerve terminals, the packaging of neurotransmitter was pharmacologically blocked in recycling vesicles and the action of the well-established modulator 5-HT that enhances synaptic efficacy at crustacean NMJ was investigated in this study.

Crustaceans have played a major contribution for investigating structure and function relationships in synaptic transmission that have aided in understanding synapses in general for all animals (Atwood, 1976, 1982a,b; Jahromi and Atwood, 1974; Atwood and Cooper, 1995, 1996a,b; Cooper et al., 1995a,b, 1996a,b; Walrond et al., 1993; Johnstone et al., 2008, 2011; Denker et al., 2011a). An advantage of many NMJs in the crayfish is that they are graded in transmission as many crustacean muscles do not produce action potentials (Atwood, 1967, 1976). This allows one to follow a rise or decrease in synaptic efficacy over time as well as influences in modulation of the synaptic function with quantal analysis (Dudel and Kuffler, 1961; Cooper et al., 1995b, 2003; Djokaj et al., 2001).

Selective axonal stimulation first studied in crayfish leg extensor yielded two different types of muscle contraction: one a fast twitch-like, the other one with a slower response but depression

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resistant (Lucas, 1907, 1917; Blaschko et al., 1931; Wiersma, 1933; Van Harreveld and Wiersma, 1936). Later the same contractile pattern and physiology was also identified in crayfish abdomen extensor and flexor musculature (Kennedy and Takeda, 1965a,b; Parnas and Atwood, 1966). Physiological and histological studies suggested that this difference is not only due to the types of motor neurons (phasic/high-output and tonic/low-output), but also the structure of postsynaptic targets (fast and slow muscles) (Baierlein et al., 2011).

The morphological and physiological differences of tonic and phasic nerve terminals have been studied in the crayfish model (Atwood, 1963, 2008) and particularly well in the leg extensor for comparisons (King et al., 1996; Bradacs et al., 1997; Msghina et al., 1998). In this leg preparation both types of nerve terminals innervate the same postsynaptic muscle fiber and give rise to stark differences in postsynaptic responses. An advantage of this preparation is that the target is the same fiber so comparisons in neuronal communication can be probed. In this preparation the small varicosities have a high mean quantal content and the synapses depress relatively quickly within the phasic terminals. However, the larger varicosities of the tonic nerve terminal have a low mean quantal content and show marked facilitation with resistance to depression (Wu and Cooper, 2010). Given that the nerve terminals in this preparation do innervate the same fiber there might be feedback from the fiber being stimulated by one neuron to the other non-stimulated neuron or an alteration in receptor sensitivity. So, for our current study we chose to use distinctly separate muscles in the crayfish abdomen that fit phasic and tonic profiles to avoid interaction of the muscle activity.

The abdominal extensor musculature has been well described in *Procambarus clarkii* and other closely related species (Pilgrim and Wiersma, 1963; Parnas and Atwood, 1966; Sohn et al., 2000). All three deep extensor muscles (medial – DEM, lateral 1 – DEL1, lateral 2 – DEL2) are composed of phasic type of muscle fibers (fast-contracting fibers) with short sarcomeres less than 5 μm (Parnas and Atwood, 1966). DEM is a twisted helix muscle. Most of the DEL1 and DEL2 muscle fibers are straight. Kennedy and Takeda (1965a,b) had identified the superficial extensors medial – SEM and lateral – SEL and their innervation profiles. Both the superficial muscles contain tonic muscle fibers (slow-contracting fibers) with longer sarcomeres ranging 9–11 μm (Parnas and Atwood, 1966) (see Fig. 1 for details).

A recent study of the tonic NMJs on the crayfish opener muscle in the walking leg did demonstrate that blocking the vesicular glutamate transporter (VGlut) with bafilomycin A1 depressed synaptic transmission faster than without its presence and that the rate of synaptic depression is stimulation dependant (Wu and Cooper, 2012a). Also, a working dose of 4 μM bafilomycin A1 was demonstrated to work well without functional damage to crayfish NMJs. The opener NMJ has a substantial RP which can be recruited by 5-HT application after the induction of synaptic depression, suggesting a functional separation in vesicles of the RRP and RP. In this study, the high-output terminals on the DEL1 muscle fibers were compared to the low-output terminals in SEL muscle fibers within the same segment of abdominal musculature. Based on previous reports of the morphological and physiological characteristics of the tonic and phasic terminals, a slower depression of the tonic terminals with or without bafilomycin A1 treatment was predicted. Also, we expected that treatments with bafilomycin A1 would depress both the terminals faster than without the drug; however, we also predicted phasic terminals would show a much greater rate of depression than tonic terminals in the presence of bafilomycin A1. Given the recent results of tonic terminals on the leg opener NMJs responding to 5-HT after the induction of depression, we expected to be able to recruit vesicles from RP in the tonic terminal to a greater extent than the phasic terminals in the abdominal preparations. Working models in the functional difference of the vesicle pools in low- and high-output terminals are presented.

2. Materials and methods

General. All the experiments were carried out in the midsize crayfish (*P. clarkii*) measuring 6–10 cm in body length. They were individually housed in plastic containers with oxygenized water. The temperature of the animal room was controlled at 20–21 °C. The animals were fed with dry fish food and water changed on weekly basis.

Dissection. The dissection is described in Sohn et al. (2000). All the connective tissues and residual flexor muscles were removed to better visualize DEL1, DEL2, DEM and SEL. Only DEL1 and SEL in segment A2, A3, A4 were used (Fig. 1). Dissected preparations were maintained in crayfish saline, a modified Van Harreveld's solution

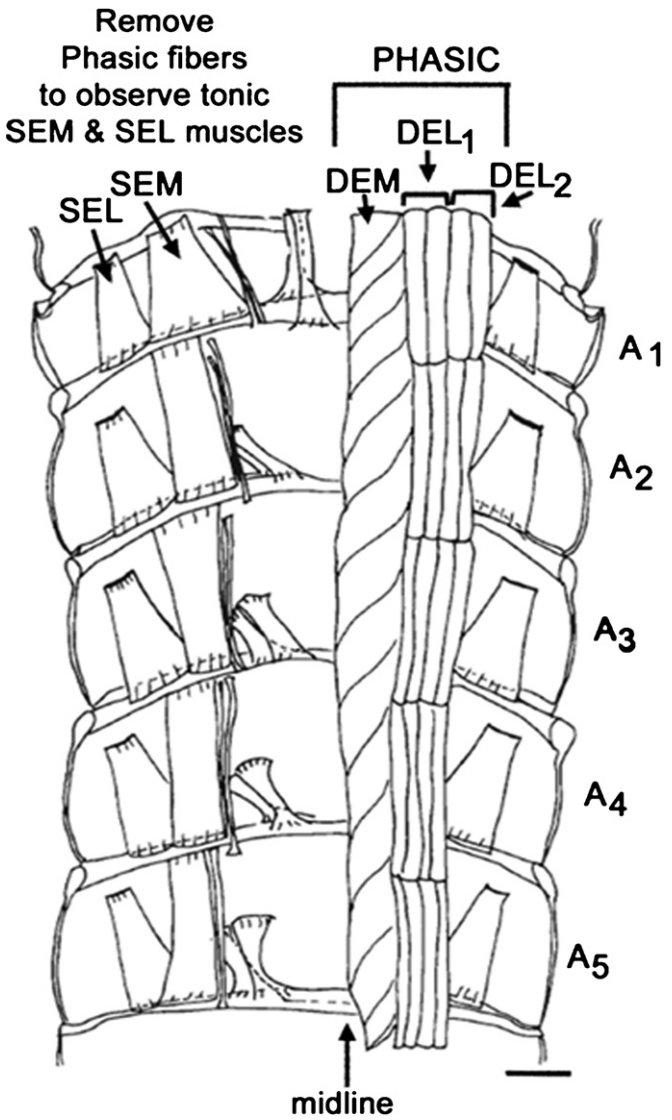

Fig. 1. Schematic presentation of crayfish abdomen extensor musculature. Each side of each segment contains deep extensor medial muscle (DEM), deep extensor lateral muscle 1 (DEL1), deep extensor lateral muscle 2 (DEL2), superficial extensor lateral muscle (SEL), superficial extensor medial muscle (SEM). On the left side of the figure, dorsal SEL and SEM are viewed by removing DEM, DEL1, and DEL2. DEM, DEL1 and DEL2 are phasic muscles whereas SEM and SEL are tonic in nature. A1–A5 refers to abdomen segments. Scale bar = 2.35 mm.
The figure is modified from Sohn et al. (2000).