The Crayfish Fast Extensor Muscle Exhibits Both NMDA and Non-NMDA Receptor Activity

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ABSTRACT

In the neuromuscular junction of the crayfish, the excitatory neurotransmitter is glutamate. There are two types of glutamate receptors, N-methyl-D-aspartate (NMDA) and non-NMDA receptors, both of which have been demonstrated to be present in the opener muscle and deep abdominal extensor muscle. The crayfish fast extensor muscle, however, is an understudied preparation whose receptor types are undocumented. Using antagonists for both NMDA and non-NMDA receptors (2-amino-5-phosphonovaleric acid, or APV, and 6-cyano-7-nitroquinoxaline-2,3-dione, or CNQX, respectively, at a concentration of $10^{-5}$ M) to inhibit the generation of excitatory junction potentials (EJPs), this study demonstrates the existence of both NMDA and non-NMDA receptors in the fast extensor muscle. Further, results indicate that antagonist binding in this preparation is easily reversible, and that the two glutamate receptor types have different receptor-ligand interactions as evidenced by differences in kinetics.

INTRODUCTION

Invertebrates such as crayfish have proven to be excellent models for the study of synaptic junctions. Much of the information we now have about glutamate receptors was discovered in work done on crayfish preparations (Dudel, et al., 1987; Shinozaki, 1988; Parnas, et al., 1994; Dudel, et al., 1997; Schramm, et al., 1997), specifically the neuromuscular junction (NMJ) of the opener muscle and deep abdominal extensor muscle. In these preparations, the excitatory junction potential (EJP) is elicited by the neurotransmitter glutamate. The opener muscle of the crayfish has been shown to have two types of post-synaptic glutamate receptors, known as N-methyl-D-aspartate (NMDA) and non-NMDA receptors (Parnas et al., 1994). In addition, both types of receptors have been found to have antagonists which are able to bind and inhibit the normal excitatory action of glutamate. While the pharmacology of the receptors has been studied extensively in the opener muscle preparation (Parnas et al., 1994; Schramm et al., 1997), the fast extensor muscle has been largely overlooked. We could find no information regarding glutamate receptor types or their kinetics in this preparation.

In this study, our objective was to demonstrate the presence of either or both types of glutamate receptors in the fast extensor muscle preparation, and to study the kinetics of those receptor-ligand interactions. For example, we hoped to characterize the binding of the antagonists (i.e. reversibility) by testing for return of the EJP after removal of the inhibitory agent. Using antagonists for both NMDA and non-NMDA receptor types, we attempted to inhibit the EJP in the muscle preparation. Our results suggest that the fast extensor muscle contains both NMDA and non-NMDA receptors. Further, the kinetics
of the two receptor antagonists suggest that their methods of action differ, as has been shown in previous studies (Shinozaki, 1988; Beneviste et al., 1991; Clements et al., 1991; McBain et al., 1994; Clements et al., 1998).

MATERIALS AND METHODS

Muscle preparation. The fast extensor muscle of crayfish (Procambarus spp.) was used. Crayfish were chilled for 15 min., and their tails removed. Fast extensor muscles were extracted as described by Stephens (1996) and placed in crayfish physiological saline solution. All measurements were taken from medial extensor muscles.

Equipment setup. Membrane potential and EJPs were measured using a standard microelectrode cell insertion method (Stephens, 1996). The innervating nerve was stimulated using a Grass SD9 Stimulator (frequency = 1 pps; delay 2.5 ms; duration = 1 ms). Stimulating voltages used were preparation dependent and suprathreshold. Measurements were recorded using the software program Scope and EJPs analyzed each represent an average of 16 sweeps.

Antagonist application and washes. Baseline membrane resting potentials and EJPs were recorded in standard physiological saline solution prior to antagonist application. The NMDA antagonist 2-amino-5-phosphonovaleric acid (APV) and non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were both diluted in saline solution to a concentration of 10^{-5} M and applied to the muscle preparation as a bath. Following loss of EJP, preparations were washed once with standard physiological saline for approximately one minute, then immersed in fresh saline solution. Measuring was then continued until the EJP was rescued. The procedure was performed twice in each preparation.

RESULTS

APV was shown to cause a decrease in the amplitude of the EJP in the crayfish fast extensor muscle following both applications of the antagonist (76% and 95% from baseline), as shown in Figure 1. We noted that the second application of APV resulted in a more rapid decrease in amplitude.

Figure 1

![Figure 1](image)
than the first, reaching minimum value after 8 minutes, while the first application reached minimum value after 24 minutes. The EJP returned following the saline washes (Fig. 1). Further, we observed that the reestablished EJPs reached amplitudes which were appreciably lower than the baseline value (46% and 58% lower). Finally, the amplitude consistently decreased over time.

CNQX demonstrated a similar effect on the crayfish fast extensor muscle, as shown in Figure 2. EJP amplitudes decreased substantially following application of the antagonist (87% and 75% from baseline), and returned after saline washes (Fig. 2). As with the APV applications, renewed amplitudes in the CNQX-treated preparations were consistently lower than the baseline value (44% and 33% lower), and overall amplitude decreased over time.

Upon comparing the time courses of the two antagonists, we noticed that application of CNQX led to more rapid inhibition and recovery of EJPs than did APV. CNQX activity was nearly twice as rapid (2.4 times) as that of APV in the first application (Fig. 1 & 2).

**DISCUSSION**

In this study we attempted to demonstrate the presence of both NMDA and non-NMDA glutamate receptors in the crayfish fast extensor muscle. Our results support the existence of both receptor types in the preparation, as evidenced by the fact that both glutamatergic receptor antagonists were able to inhibit the EJP. While the amplitude of the EJP during immersion in the antagonist solutions never actually reached zero, we consider the substantial decreases in amplitude shown in Figures 1 and 2 to be indicative of inhibition.

The inhibitory ability of both APV and CNQX was further supported by the reversal of the inhibition after removal of the antagonists. Washing of the muscle preparation with standard physiological saline solution following the antagonist applications led to a gradual return of the EJP, an effect known as rescue. Our observation of this effect supports previous findings that the antagonists bind reversibly to their respective receptors (Shinozaki, 1988). Further, the replication of this
rescue in both preparations indicates that the inhibition of the EJP which we observed was in fact due in large part to the glutamate receptor antagonists, and not solely to extrinsic factors such as deterioration of the muscle preparation.

Though differences in amplitude between antagonist and wash periods were clearly evident, we did observe an overall continual decrease in amplitude over time in both preparations. While the saline washes in both preparations led to rescue, the first rescue amplitudes were considerably smaller than the baseline values, and the second rescue amplitudes were smaller than the first (Fig. 1 & 2). We believe that this effect is attributable both to deterioration of the muscle preparation over time, and more importantly, to residual antagonist molecules in the saline solution that persisted despite washing. This ability of residual molecules to inhibit the EJP provides preliminary information about the antagonist concentrations necessary to achieve inhibition. Despite this overall decrease over time, however, we believe that these results conclusively demonstrate the presence of both glutamate receptor types in the crayfish fast extensor muscle.

As discussed above, it appears that the two antagonists bind reversibly to their receptors, and that their inhibitory effects may be concentration dependent. In order to evaluate the action and kinetics of the two antagonists, we looked closely at the time courses of inhibition and rescue. We noticed that the NMDA-receptor antagonist APV exhibited a slower rate of inhibitory action and of EJP rescue than did the non-NMDA antagonist CNQX. In fact, CNQX activity was nearly twice as rapid as that of APV, and rescue of the EJP in the CNQX preparation was observed to be nearly instantaneous, though we were unable to capture this data due to technical problems. These differences in the kinetics of the two antagonists and their receptors indicate that their methods of action are different, as has been supported in previous studies of the glutamate receptor subtypes (Shinozaki, 1988; Edmonds, et al., 1995). For instance, it has been determined that NMDA receptors have slower kinetics than do non-NMDA receptors (McBain et al., 1994). These differences in kinetics are partially explained by the findings of Clements, et al. (1998) who demonstrated that non-NMDA receptors bind two ligand molecules, and Benveniste, et al. (1991) and Clements, et al. (1991) who showed that NMDA receptors require the binding of four molecules. Our kinetics data for both receptor types concur with these previous findings. We believe, however, that more data are necessary to understand the action of both receptors in the fast extensor muscle preparation.

In conclusion, we have demonstrated the presence of both NMDA and non-NMDA glutamate receptors in the fast extensor muscle of the crayfish. Further, the kinetics we observed support previous findings that the binding of the receptor antagonists APV and CNQX is reversible, and that these receptor subtypes function using different mechanisms.

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