Effects of Calcium Antagonist Drugs on Acetylcholine and High K Responses of a Molluscan Muscle *Neptunea Antiqua*

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Abstract. The odontophore retractor (OR) muscle of *Neptunea antiqua* depends on external calcium to raise internal cellular calcium concentration of the contractile system to activation level. The acetylcholine (Ach) – and high K – induced responses were abolished in nominally Ca-free and 2 mmol/l EGTA/Ca/free salines. Diltiazem and nifedipine were inhibitory on both Ach and high K responses of the OR muscle. However, verapamil was inhibitory only on K but not Ach response. Compared with the radular retractor and radular sac muscle, the OR muscle of *Neptunea* shows some differences but also similarities in its slow calcium channel activities even though all these muscles are bound together in the same organism.

Key words: Acetylcholine — Diltiazem — Nifedipine — Verapamil — Molluscan muscle — Calcium — *Neptunea Antiqua*

Introduction

The proboscis muscle of *Busycon canaliculatum* (Huddart and Hill 1988; Huddart et al. 1990 a,b; Brooks et al. 1990), *Buccinum undatum* and *Neptunea antiqua* (Alohan 1991, 1993) require extracellular calcium for activation of their contractile systems. Recent studies of these muscles have shown a great diversity in their responses to acetylcholine and elevated potassium salines (Huddart et al. 1990 a,b; Alohan 1993). Previous studies of the radular sac and radular retractor muscles of the proboscis of *Neptunea antiqua* (Alohan 1993) show that there are differences as well as similarities in their calcium dependency even though they are bound together in the same proboscis, and function to complement each other in the over all feeding mechanism of the radula.

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In this study, the Ach and high K responses of the odontophore retractor muscle of *Neptunea* are investigated, firstly to characterise its calcium modulation, and secondly to establish whether or not there are differences or similarities in the calcium modulation of this muscle and the other muscles which comprise the proboscis.

Materials and Methods

Specimens of *Neptunea antiqua* were obtained from Scottish Marine Biological Association, Millport, and kept in aerated sea water aquaria at 16 °C. The odontophore retractor muscle (OR) was dissected from the proboscis sheath exactly as described for the equivalent *Busycon canaliculatum* (Huddart et al. 1990a). To record tension, the muscle was ligated with monofilament nylon and suspended in 10 ml jacketed organ baths between the hook and Grass FT.03 force displacement transducers. The organ baths were constantly aerated, and a passive load of 1 g was applied to the muscle. Force displaced by the muscle was recorded on a four-channel Grass 7D polygraph. High-potassium depolarization of the muscle was achieved by direct application of appropriate amounts of 3 mol/l KCl stock solution. Responses obtained in this way did not differ from those obtained with equimolar K^+/Na^+ substitution (Huddart and Hill 1988). Acetylcholine was applied to the preparation by direct addition of appropriate amounts of 10 mmol/l stock solution made up in sea water. Diltiazem was applied from freshly prepared stock solution in distilled water. Verapamil and nifedipine were made up as 25 mmol/l stock solution in ethylalcohol as solvent. The final bath solvent level did not exceed 0.1% which in control trials was found to be without effect on the preparation (Huddart and Hill 1988). Experiments with verapamil and nifedipine were carried out in subdued lighting because of the photosensitivity of these compounds. All experiments were carried out at room temperature (15- 20 °C). After setting up, the preparations were allowed to equilibrate to bath conditions for 30 min, and a standard time interval of 20 min was allowed between drug exposures. Each experiment was replicated four times.

Results

Dependency upon extracellular calcium

The odontophore retractor (OR) muscle developed prompt responses to 100 mmol/l K and 5×10^{-6} mmol/l Ach (Fig. 1*A*, *C*). The responses were found to be dependent on external calcium. When exposed to nominally calcium-free saline for 15 min, the responses to high-K and Ach salines were both abolished (Fig. 1*B*, *D*). Recovery of both Ach and K responses after 30 min return to normal sea water was complete. In calcium-free 2 mmol/l EGTA salines, both Ach and high-K responses were also abolished (Fig. 2*B*, *E*). Recovery of the responses after 20 min return to normal sea water was almost complete in high-K saline (Fig. 2*C*) and about 50% in Ach saline (Fig. 2*F*).

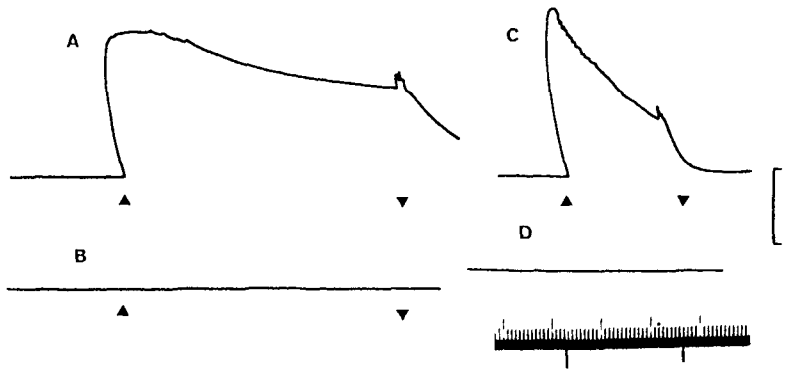

Figure 1. Responses of *Neptunea* odontophore retractor (OR) muscle to 100 mmol/l K sea water and 5×10^{-6} mol/l Ach Treatments *A*, control K contracture, *B*, K contracture after 15 min in calcium-free sea water, *C*, control Ach contracture, *D*, Ach contracture after 15 min in calcium free sea water Time (1 min major marks) and tension (1 g) calibrations apply to all traces

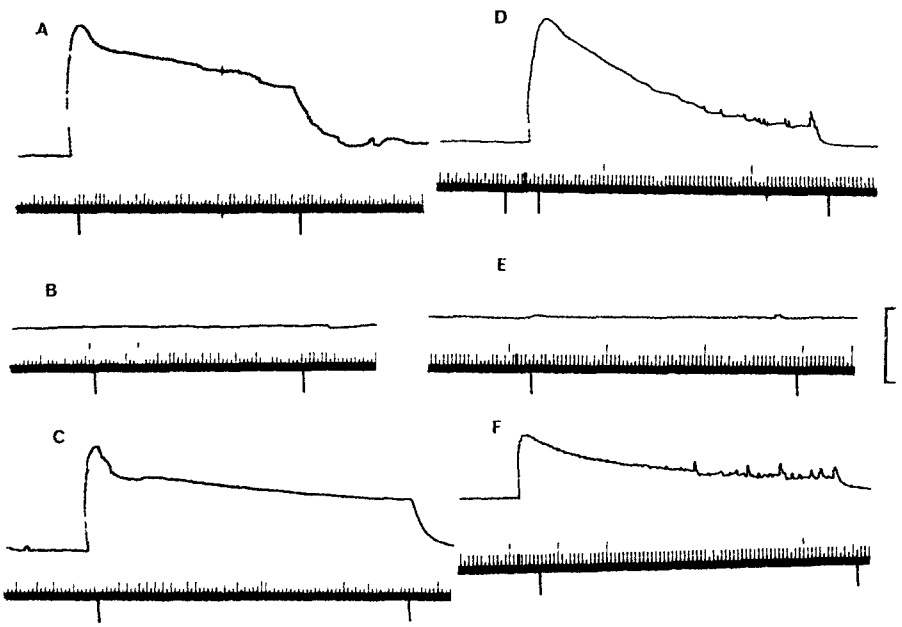

Figure 2. The effect of EGTA on 100 mmol/l K contracture and 5×10^{-6} mol/l Ach contracture of OR muscle Treatments *A*, control K contracture *B*, K contracture after 15 min in calcium-free 2 mmol/l EGTA saline *C*, K contracture after 20 min return to normal sea water, *D*, control Ach contracture, *E*, Ach contracture after 15 min in calcium free 2 mmol/l EGTA saline *F*, Ach contracture after 20 min return to normal sea water Time (1 min major marks) and tension (1 g) calibrations apply to all traces

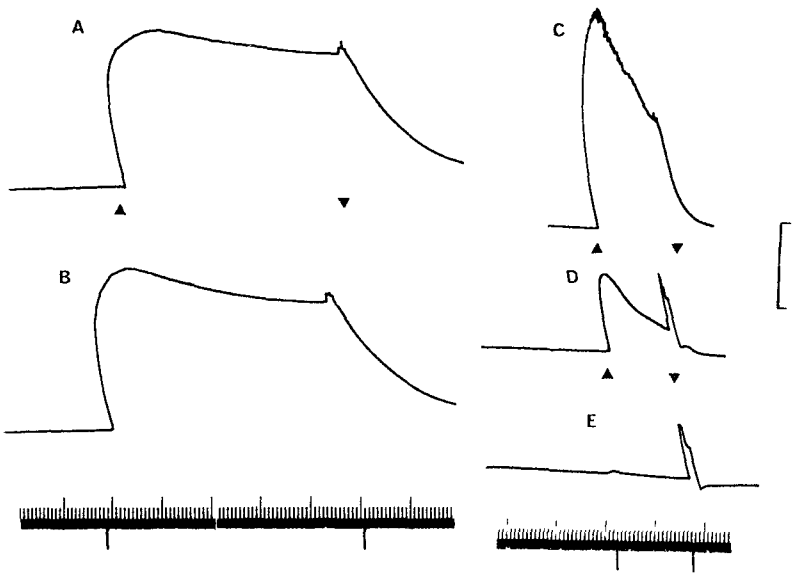

Figure 3. The effect of diltiazem on 100 mmol/l K contracture and 5×10^{-6} mol/l Ach contracture of OR muscles. Treatments: *A*, control K contracture; *B*, K contracture after 15 min perfusion with 10^{-5} mol/l diltiazem, *C*, control Ach contracture; *D*, Ach contracture after 15 min perfusion with 10^{-5} mol/l diltiazem; *E*, Ach contracture after 15 min perfusion with 5×10^{-5} mol/l diltiazem. Time (1 min major marks) and tension (1 g) calibrations apply to all traces.

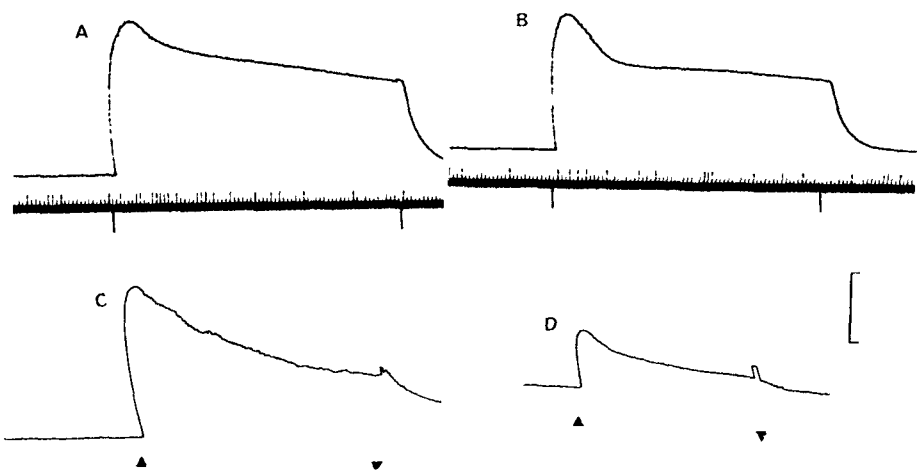

Figure 4. The effect of nifedipine on 100 mmol/l K contracture and 5×10^{-6} mol/l Ach contracture of OR muscles. Treatments: *A*, control K contracture; *B*, K contracture after 15 min perfusion with 10^{-5} mol/l nifedipine; *C*, control Ach contracture; *D*, Ach contracture after 15 min perfusion with 10^{-5} mol/l nifedipine. Time (1 min major marks) and tension (1 g) calibrations apply to all traces

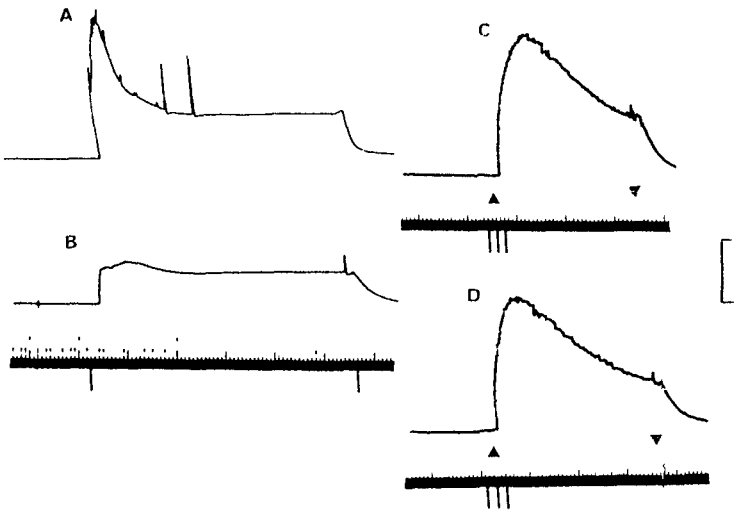

Figure 5. The effect of verapamil on 100 mmol/l K contracture and 5×10^{-6} mol/l Ach contracture of OR muscle. Treatments: *A*, control K contracture; *B*, K contracture after 15 min perfusion with 10^{-5} mol/l verapamil; *C*, control Ach contracture; *D*, Ach contracture after 15 min perfusion with 10^{-5} mol/l verapamil. Time (1 min major marks) and tension (1 g) calibrations apply to all traces.

The effect of calcium antagonists

The organic calcium antagonist drugs diltiazem, verapamil and nifedipine were employed to examine further the calcium dependency of Ach and high-K responses of OR muscle. At 10^{-5} mol/l, diltiazem was without effect on high-K-induced response of OR muscle (Fig. 3*B*). Diltiazem, at 10^{-5} mol/l, inhibited Ach-induced response by more than 50% (Fig. 3*D*) while at 5×10^{-5} mol/l, it completely inhibited the Ach response of OR muscle (Fig. 3*E*). Nifedipine at 10^{-5} mol/l was without effect on K-induced response (Fig. 4*B*) but the Ach-induced response of OR was inhibited (Fig. 4*D*).

Verapamil, at 10^{-5} mol/l, substantially inhibited the K-induced response (Fig. 5*B*) while it was without effect on Ach-induced response of OR muscle (Fig. 5*D*).

Discussion

The OR muscle of *Neptunea* proboscis depends on $[Ca]_0$ in its response to Ach and high-K salines. The responses were inhibited in calcium-free sea water, indicating that Ach and high-K responses of OR muscle depend upon an inward movement

of extracellular calcium to activate the contractile system. This view is supported by exposure of the OR muscle to 2 mmol/l EGTA/calcium-free sea water in which both Ach- and high-K-induced contractures were also totally inhibited. In the absence of EGTA, a calcium chelator, recovery of the Ach and high-K response in OR muscle was more rapid, hence EGTA may have depleted internal calcium pools which required refilling. The lack of external calcium induced electro-mechanical uncoupling in the OR muscle and since there was no depolarization in calcium-free conditions, this suggests that it is calcium influx which mediated both Ach and high-K depolarization. This is in accord with the external calcium dependency of the radular sac and radular retractor muscles of *Neptunea* (Alohan 1993) and other molluscan muscles (Huddart and Hill 1988; Huddart et al. 1990b; Brooks et al. 1990). However, the possibility exists that Ach may cause release of calcium from submembrane vesicles in the molluscan muscle by induction of a secondary messenger while high-K saline may cause the release of calcium-induced release of calcium (Tameyasu and Sugi 1976; Brooks et al. 1990).

Unlike mammalian and insect skeletal muscles with well organized T-system and sarcoplasmic reticulum (SR) which store intracellular calcium, the molluscan muscles possess membrane vesicles (Sanger and Hill 1972, 1973; Dorsett and Roberts 1980; Hunt 1981) which are regarded as equivalent to SR of skeletal muscles even though they lack the calcium storing capacity (Sugi and Atsumi 1973; Suzuki and Sugi 1978). These vesicles are thought to act as loci for the release of activator calcium required for activating the contractile system in OR muscle of *Neptunea.*

Diltiazem, nifedipine and verapamil are regarded as antagonists of the slow voltage-independent calcium channel which is long-lasting, not readily inactivated and operating at substantial levels of membrane depolarization in mammalian muscle (Van Breemen et al. 1981; Carbone and Lux 1984; Glossman et al. 1984; Sperelakis 1984). The Ach-induced responses of OR muscles show sensitivity to diltiazem and nifedipine but the K-induced responses were relatively unaffected by both calcium antagonists. In OR muscle, these drugs have effectively uncoupled Ach-induced excitation from contraction by blocking calcium influx. So it is probable that Ach responses of OR muscle of *Neptunea* depend upon slow calcium channel activity. The effect of diltiazem and nifedipine seen in the OR muscle is in agreement with their inhibitory effect in OR and RS muscles of *Buccinum* (Alohan 1991) but contrasts with their insensitivity in RR and RS muscles of *Neptunea* (Alohan 1993) on the one hand and their excitatory effect on *Busycon* proboscis muscles (Huddart and Hill 1988; Huddart et al. 1990b) on the other. Diltiazem and nifedipine had no effect on K-induced contractures of OR muscle of *Neptunea* even though both calcium antagonists were inhibitory on the K responses of the RR and RS muscles (Alohan 1993). Paradoxically, verapamil inhibited or reduced the K response of OR muscle of *Neptunea* but had no effect on the Ach-induced

response (Fig. 5). In the RR and RS muscle, however, verapamil inhibited the Ach-induced responses (Alohan 1993).

Thus the slow calcium channel in the OR muscle shares similarities with those present in the RR and RS muscles of *Neptunea* proboscis although there are also differences in all these muscles which comprise the proboscis. What has emerged from this study is: that there is a diversity in responses of the OR, RR and RS muscles of the same proboscis in *Neptunea* to the calcium antagonists; that the differences may reflect the different functional roles which each of these muscles performs in the overall rasping mechanism of the radula.

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